Effect of organic and conventional agronomic practices and variety choice on nutritional quality, the contents of undesirable compounds and yield of cereals

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Abstract

Cereal products are one of the most important sources of nutrients and energy in the human diet, and common wheat is the most consumed crop globally. Spelt wheat -one of the most ancient cereals- is increasing its share in the food markets because of its ability to grow under low inputs and consumers' belief about its high quality. The demand for spelt wheat and other minor cereals is particularly high in the organic food market, much of which is driven by consumers' expectations that organic farming practices could improve the content of beneficial nutrients and decrease the content of undesirable compounds such as pesticides. The aim of this thesis was to explore the effect of organic and conventional agronomic practices (fertilisation and irrigation) and variety choice on the nutritional quality and undesirable compounds (heavy metals, mycotoxins and pesticides) of grain/flour of different cereal species (mainly common wheat and spelt wheat). The objectives were to carry out (1) a meta-analysis of data on effects of organic and conventional agronomic practices on mycotoxin contamination in cereals and (2) a shopping basket study to collect flour from supermarkets in the UK and Germany over three years; and (3) to carry out a field experiment, where various spelt wheat genotypes were cultivated under different fertility treatment and irrigation regimes. The contents of nutritionally relevant compounds such as phenolic acids, flavonoids, protein, and macro- and micronutrients, as well as undesirable compounds such as heavy metals were measured in the shopping basket and field study. In addition, mycotoxins and pesticide residues were measured in the shopping basket study.

The meta-analysis of mycotoxin content of cereal grains was based on 79 published studies and found that, historically, conventional cereals had consistently higher levels of *Fusarium* mycotoxin contamination and organic cereals had higher levels of OTA contamination. However, the contamination and prevalence of OTA in organic cereals has decreased in cereal grains/products in the last 15 years in Europe due to the improvement of post-harvest drying and storage management. Results of the **shopping basket study** found that antioxidant capacity, concentrations of phenolic phytochemicals and mineral micronutrients were significantly higher in organic and wholegrain flours compared with conventional and white common wheat and spelt wheat flour, repectively, while conventional wholemeal flour was contaminated with significantly higher pesticide residues than conventional white flour. These results suggest that switching to organic wholemeal flour allows for higher intakes of phenolic phytochemicals and mineral micro-nutrients. These have been associated with potential health benefits of consuming wholegrain foods to be achieved without simultaneously increasing dietary exposure to pesticides. No differences in mycotoxin contamination were found between conventional and organic

farming systems. Results of a **controlled experiment** in Crete indicated that, supplementary irrigation substantially improved grain yield of spelt but had no negative effects on mineral and phenolic phytochemical content, and sheep and chicken manure fertilisation resulted in similar yields as mineral fertilisation which emphasised the suitability of spelt for organic production in semi-arid conditions. No major impact of fertiliser type was seen, but a significant impact of spelt variety was found on concentrations of phenolic phytochemicals and some minerals.

The main achievements and novelty of the project were

- carrying out the most comprehensive meta-analysis to date of mycotoxin contamination in organic vs conventional cereals;
- carrying out an extensive and comprehensive investigation of the nutritionally relevant compounds and the content of undesirable compounds found in organic vs conventional common wheat and spelt wheat flours available in supermarkets in the UK and Germany, predicting the potential health effects for consumers switching to organic from conventional cereals consumption, which enables 11%, 16% and 30 % more phenolics, Fe and Zn intake and at least 4 times lower pesticide intake respectively, and switching to spelt from common wheat enable 2 times higher Zn intake.
- carrying out the first assessment of the yield and grain quality performance of different spelt varieties with different irrigation management and fertility management in a semi-arid region of the Mediterranean; when taking both yield and grain quality, the “organic” spelt variety ZOR was recommended together with sheep or chicken manure fertilisation and sustainable drip irrigation.

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List of abbreviations

Species (General)	
Spelt	Spelt wheat
Wheat	Common wheat (Chapter 1, 2 and cited publication) Common wheat and spelt wheat (Chapter 4)

Meta-analysis	
GRADE	Grading of Recommendations, Assessments, Development and Evaluation
MA	Meta-analysis
MD	Mean difference
OR	Odds ratio

Study type for Meta-analysis	
St	Study type
SBS	Shopping basket study
CF	Comparison of farm study
EX	Controlled experiment study

Climate classification for Meta-analysis	
Clim	Climate
BSk	Arid, steppe, cold weather
Cfa	Code weather without dry season and with hot summer
Cfb	Temperate weather without dry season and with warm summer
Csa	Temperate weather with dry and hot summer
Cwa	Temperate weather with dry winter and with hot summer
Dfb	Cold weather without dry season and with warm summer
Dfc	Cold weather without dry season and with cold summer
Dsa	Cold weather with dry summer and with hot summer
Dwa	Cold weather with dry winter and with hot summer
Mix	More than one climate type

Analytical method for Meta-analysis	
Met	Analytical method
ELISA	Enzyme-linked immunosorbent assay
GC-MS	Gas chromatography
HPLC	High-performance liquid chromatography
LC-MS/MS	Liquid chromatography-mass spectrometry/tandem mass spectrometry

Country (Chapter 2 Meta-analysis)	
Ct	Country
AT	Austria
BE	Belgium
CA	Canada
CH	Switzerland
CZ	Czech

DE	Germany
DK	Denmark
ES	Spain
FI	Finland
FR	France
GB	United Kingdom of Great Britain and Northern Ireland
HR	Croatia
IT	Italy
KR	Korea, Republic of
LT	Lithuania
LV	Latvia
Mix	mixed countries
NL	Netherlands
NO	Norway
PL	Poland
PT	Portugal
RO	Romania
SI	Slovenia
SK	Slovakia
TR	Turkey
US	United States of America

Mycotoxin (Chapter 2 Meta-analysis and Chapter 4 Basekt Study)

15-AcDON	15-Acetyl-Deoxynivalenol
3-AcDON	3-Acetyl-Deoxynivalenol
AF	Total aflatoxins, include aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2)
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
DAS	Diacetoxyscripenol
DON	Deoxynivalenol
ERG	Ergosterol
FB1	Fumonisin B1
FB2	Fumonisin B2
FUM	Fumonisins
FUS	Fusaproliferin
FUX	Fusarenon X
MAS	Monoacetoxyscirpenol
NIV	Nivalenol
OTA	Ochratoxin A
ZEA	Zearalenone

Antioxidant related (Chapter 4 Shopping basket study and Chapter 5 Field experiment)

PC	Phenolic content
DW	Dry weight
FLA	Flavonoid
FRAP	Ferric reducing antioxidant power
TEAC	Trolox equivalent antioxidant capacity
TPC	Total phenolic content

Minerals (Chapter 4 Shopping basket study and Chapter 5 Field Experiment)

Al	Aluminium
As	Arsenic
Ca	Calcium
Cd	Cadmium
Cr	Chromium
Cu	Copper
Fe	Iron
Hg	Mercury
K	Postassium
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Na	Sodium
Ni	Nickel
P	Phosphorus
Pb	Lead
S	Sulfur
Se	Selenium
Zn	Zinc

Quality Control related

CODEX	CODEX ALIMENTARIUS
EU	European Union
FAO	Food and Agriculture Organization
MCL	Maximum contamination levels
MRL	Maximum residue levels
QA	Quality assurance
ROSA	Rapid One Step Assay
USDA	United Stated Department of Agriculture

Pesticide (Chapter 4 Shopping basket study)

CCC	Chlormequat
FHB	*Fusarium* head blight
OP	Organophosphate
PBO	Piperonyl butoxide
CPP	Crop protection product

Statistical Analysis and Results in tables related (Chapter 4 and chapter 5)

ANOVA	Analysis of variance
BR	Bran
CHI	Chicken manure
CONV	Conventional
CT	Country
FIL	Filderstolz
FS	Farming System
FT	Fertiliser type
GS	Growth stage
IR	Irrigation
LOD	Limit of detection
LOQ	Limit of quantification
MIN	Mineral fertiliser
NS, ns	Not significant
OBE	Oberkulmer
ORG	Organic
RUB	Rubiota
SD	Standard Deviation
SE	Standard Error
SHE	Sheep manure
SP	Species
SPAD	Leaf chlorophyll
SV	Species variety
TGW	Thousand grain weight
YR	Year
ZOR	Zürcher Oberländer Rotkorn

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Chapter 1 Introduction

1.1 Organic farming and agronomic practice differences from conventional system

1.1.1 Introduction to organic farming

Organic farming has dramatically grown in the last decade across the world (Paull, 2011). The value of organic retail sales in Europe increased from 10.2 billion in 2004 to 29.8 billion in 2015 and the area of organic agricultural land in European went up to 12.7 million hectares in 2015 from 1 million hectares in 1994 (Statista, 2016). The most recent report by the Soil Association (2018) indicated that, during 2016 to 2017, the percentage of organic food in the total market increased at a rate of minimum 7.1% in the UK, maximum 20% growth in Denmark (Soil Association, 2018). The increasing number of consumers choosing organic products may be due to their perception that (a) there is a lower risk of exposure to undesirable nutritional compounds including pesticide residues, mycotoxins, and heavy metals because the use of pesticides and mineral fertilisers is prohibited in organic production farming systems, and (b) there are higher concentrations of nutritionally desirable phenolic phytochemicals, which are perceived by consumers as being healthier, and additionally (c) animal welfare and environment sustainable development issues get more attention from the public (Lee and Yun, 2015; Yadav and Pathak, 2016; Apaolaza *et al.*, 2018).

1.1.2 Difference of agronomic practices between organic and conventional farming systems

Conventional Farming

The intensification of agricultural practices (often also described as the green revolution) applied in conventional farming system over the last 40-50 years involved (a) the introduction of new varieties with higher yield potential (in cereals this was based on the introduction of semi-dwarfing genes to reduce stem length and increase harvest index), (b) introduction of novel irrigation technology and an expansion of irrigated production areas, (c) an increased use of nitrogen, phosphorus, potassium (N, P and K) fertilizers and (d) the introduction of a wider range and more extensive use of synthetic chemical pesticides, soil sterilants and growth regulators (Rekha *et al.*, 2006; Aktar *et al.*, 2009). More recently the intensive use of mineral fertilization and synthetic chemical pesticides (including herbicides, insecticides, nematicides and fungicides), although highly effective in improving yields and controlling weeds, pests and diseases, was linked to a range of negative side effects. These included (a) unintended negative effects on a range of non-target organisms/wildlife (including natural enemies of pests), (b) pollution of ground and drinking water (Rekha *et al.*, 2006; Aktar *et al.*, 2009), (c) negative effects on human health from environmental and food-intake based on

exposure to pesticides and including birth defects, reduced fertility, damage to the nervous system and cancer (Alavanja *et al.*, 2004; Rekha *et al.*, 2006; Aktar *et al.*, 2009; Nicolopoulou-Stamati *et al.*, 2016) and (d) negative effects on soil communities and community composition (Geisseler and Scow, 2014) and the loss of 50-100 billion tons of soil organic carbon worldwide over the past 200 years resulting from conversion of natural to agricultural land (Jarecki and Lal, 2003).

Organic farming

Organic farming standards prohibit the use of chemosynthetic pesticides and water soluble mineral nitrogen, phosphorus and potassium fertilizers and restrict the use of other mineral fertilizers. Disease control therefore relies on the use of (a) diverse rotations, (b) more resistant varieties and (c) fertilization regimes based on green and animal manures which avoid excessive N-availability and associated increase in disease susceptibility. In many regions of Europe “organic” wheat cultivars (varieties developed for and selected under organic farming conditions) are now available, resulting in different cultivars being used in organic and conventional farming systems.

Use of livestock and agricultural waste is the major method used throughout history to maintain soil fertility, also these are also the methods being developing for organic systems. Evaluation of the agricultural impact on soil carbon sequestration emphasizes the return of carbon (Smith *et al.*, 2000). The manure-based fertilisation of organic farming systems involves the addition of large quantities of carbon in addition to the nutrition elements with which the crops are fertilised (Fließbach *et al.*, 2007). In addition, biological parameters of soil quality were generally enhanced in organic farming systems compared with conventional systems, and partly they were positively affected by the application rate of manure in organic farming systems (Fließbach *et al.*, 2007). Furthermore, manure management within organic rotations has been shown to have a great effect on both yield and product quality.

1.2 Effect of farming system on nutritional quality of cereals and human health

More recently, the increase in consumer demand for organic foods and the development of organic agriculture (Dimitri and Greene, 2000; Escarnot *et al.*, 2012) triggered a wide range of studies to investigated the effects of agronomic management practices (organic and conventional) on (a) nutritional quality of crop plants from the mid-1990’s onwards and more recently (Woese *et al.*, 1997; Worthington, 2001b; Hussain *et al.*, 2012; Smith-Spangler *et al.*, 2012a; Baranski *et al.*, 2014; Vrcek *et al.*, 2014), (b) epidemiological studies aimed at identifying associations between organic food consumption and health (Huber *et al.*, 2011; Barański *et al.*, 2017) and (c) dietary intervention studies aimed at identifying the impact of

organic feed consumption on physiological health related parameters/markers in animal models (Phillips and Hart, 1935; Skwarlo-Sonta *et al.*, 2011). However, many uncertainties remain related to both (a) the impact of organic production methods on food composition and (b) potential impacts of organic food consumption on human health. Cereals/cereal-based food products have been the basis of the human diet for a long time. They are an excellent source of minerals, vitamins, and other micronutrients required for adequate health (Borneo and Len, 2012). Therefore, it is of importance identifying the nutritional performance as well as undesirable components of cereals/cereals-based food under organic and conventional management to understand the impact of production methods on food composition and the potential of whether there are health benefits through shifting to organic cereals consumption from conventional ones.

1.2.1 Phenolic phytochemicals and antioxidant capacity

Phenolic phytochemicals are secondary metabolites of plants, naturally occurring in fruits, vegetables, and cereals. Based on the number of phenol rings, phenolic phytochemicals are classified into four groups: phenolic acids; flavonoids; stilbenes and lignans. Phenolic phytochemicals, because of their ring structure demonstrate antioxidant properties *in vitro* and so are often classified as phenolic antioxidants. Their role in the body, however has been difficult to establish, in particular whether they continue to react as antioxidants, and if this confers a health benefit. The distribution of phenolic phytochemicals in plants at the tissue, cellular and subcellular levels is not uniform. For example, insoluble phenolic phytochemicals are found in the cell wall, while soluble phenolic phytochemicals are present within the plant cell vacuoles (Stewart *et al.*, 2001; Kesarwani *et al.*, 2014). Phenolic phytochemical compounds are not essential nutrients for humans but there is strong scientific evidence for health benefits associated with increased consumption of crops rich in phenolic phytochemicals, as they may play a role in preventing some chronic diseases (Mie *et al.*, 2017). The most recent review by Williamson (2017) stated that there is substantial data from intervention studies, mechanistic *in vitro* data and epidemiological evidence showing that a diet high in phenolics-rich fruit, vegetable and other food protects against developing cardiovascular disease and type 2 diabetes. In addition, cardio-protective, anti-cancer, anti-diabetic, anti-aging and neuro-protective effects of phenolic phytochemicals were been suggested (Pandey and Rizvi, 2009). What's more, phenolic acids including caffeic acid, gallic acid, ferulic acid and others, accounting for about one third of the phenolics phytochemicals in the human diet and found in all plant materials (Pandey and Rizvi, 2009) also have various health benefits. For example, ferulic acid is a major compound among phenolic acids found in wheat varieties and bound to the cell wall of plants (Harris and Hartley, 1976; Graf, 1992; Vaher *et al.*, 2010). It may serve an important antioxidant function in preserving physiological

integrity of cells exposed to both air and impinging UV radiation (Graf, 1992). In addition, the presence of ferulic acid or similar phenolic compounds greatly reduces free radical damage in neuronal cell systems without causing cell death themselves (Kanski *et al.*, 2002; Srinivasan *et al.*, 2007).

To measure the phenolic phytochemicals and other antioxidants, a large variety of testing methods and various analysis tools have been proposed and employed, including spectrometry, electroanalytical methods, and chromatography (Pisoschi and Petre Negulescu, 2012). Chromatographic methods were often applied to antioxidant separation and detection, and used before spectrophotometric determination of the total antioxidant capacity. Spectrometric techniques rely on the reaction of a radical, radical cation or complex with an antioxidant molecule capable to donate a hydrogen atom. These spectrometric techniques are indirect methods based on the reduction of persistent radicals (e.g. TEAC and DPPH), or of inorganic oxidizing species (e.g. FRAP and Folin-Ciocalteu assays) (Amorati and Valgimigli, 2014).

Prior *et al.* (2005), Amorati and Valgimigli (2014) and Pisoschi and Petre Negulescu (2012) have discussed the advantages and shortcomings of the antioxidant assessment methods. For example, the DPPH assay does not measure thiol antioxidants, such as glutathione. The FRAP assay is characterized by fast kinetics (4-6 min) but in fact, this is not always true. Some polyphenols react more slowly and require longer reaction times for detection, for example up to 30 min. However, determination replying on photometric measurements (DPPH, ABTS and FRAP assays) are simple and rapid and need only a UV-Vis spectrophotometer to perform, which probably explains their widespread use in antioxidant screening, and they still can used for preliminary screening purposes (Amorati and Valgimigli, 2014). In addition, Prior *et al.* (2005) also concluded that Folin-Ciocalteu assays and TEAC assay should be standardized for use in the routine quality control and measurement of antioxidant capacity of dietary supplements. Therefore, these methods are still valid but concerns need to be raised to some extent about the reported 'concentrations' found in the literature, and it is for this reason why some of the results are referred to as 'estimated concentrations' of the parameter assessed in some publications.

A range of studies have shown that both the composition and concentration of phenolics, other antioxidants and/or total antioxidant capacity is affected by agronomic practices in farming systems (Kalinova and Vrchotova, 2011; Almuayrifi, 2013; Beleggia *et al.*, 2013). Most recent meta-analysis identifying the composition difference between organic and conventional crops based on 343 peer-reviewed publications indicated that the concentrations of a range of antioxidants were substantially higher in organic cereals/cereal-based foods (Baranski *et al.*, 2014). There are still some studies showing significantly higher (or trends towards higher)

concentrations of phenolics and other antioxidants in organically produced cereals compared with those produced conventionally (Mäder *et al.*, 2007b; Kalinova and Vrchotova, 2011; Fares *et al.*, 2012; Kesarwani *et al.*, 2013). However, some other studies gave opposite results (Dimberg *et al.*, 2005; Gasztonyi *et al.*, 2011; Konopka *et al.*, 2012a). Therefore, there is still a need for studies focusing on specific cereals and across different crops to understand the effect of farming systems on the composition and concentration of phenolics under various environment conditions/climate.

1.2.2 Crude protein and mineral elements

Protein is an essential nutrient and cereals, as a staple food, are important sources of protein for the human diet, also the level of protein in cereal is important for bread baking (Færgestad *et al.*, 2000; Shewry, 2007). The true quantitative protein value for a food is determined by the total amount of all amino acids present in the food. However, crude protein, usually estimated by multiplying nitrogen (N) content by a nitrogen to protein conversion factor, is more common to assess the protein content of a natural product. In most food, amino-N accounts for approximately 16% of the protein weight. Hence, the nitrogen to protein conversion factor is usually 6.25 (Simonne *et al.*, 1997). Cereals contain about 6-15% protein. Results obtained by some studies showed significantly lower protein content in organic compared with conventional wheat due to lower nitrogen input in organic farming systems (Rembiałkowska, 2007; Baranski *et al.*, 2014; Vrcek *et al.*, 2014) while some studies did not detect a significant difference in protein between organic and conventional cereals (Abedi *et al.*, 2010). However, grain protein content of organic wheat could be increased by improving fertility management, choosing a legume fodder crop as the preceding crop and breeding varieties for organic farming systems (Casagrande *et al.*, 2009). It was shown that protein digestibility was higher in organic wheat flour and the quality better when measured by essential amino acid content (Krejčířová *et al.*, 2007; Vrcek *et al.*, 2014).

Minerals are divided into macro minerals, which are needed in the diet in large amounts (e.g. Ca, Mg, and K), and micro minerals or trace elements, which are required in smaller quantities (e.g. Cu, Zn, Fe, Se etc.) (Martínez-Ballesta *et al.*, 2010; Vrcek *et al.*, 2014). All body processes depend upon the action of minerals, for example, to activate enzymes performing metabolic function. Zn has been shown to be essential for intracellular metabolism and cellular growth and differentiation; it is also involved in gene expression regulation; Fe participates in oxygen and energy metabolism; and Mg is involved in the stabilization of ATP and other molecules and is also a cofactor of enzymatic systems (Ruibal-Mendieta *et al.*, 2005). Cu is an essential catalytic cofactor for selective oxidoreductases; K is a very significant body mineral, important to both cellular and electrical function (Hashimoto *et al.*, 1987). Ca is important in developing strong bones (Stipanuk and Caudill, 2013). P together with Ca form

bone structure, and play an important role in energy production (ATP), cell membranes conformation (phospholipids) and as a buffering agent (maintenance of osmotic pressure) (Li *et al.*, 2016). Deficiency of minerals may lead to various chronic diseases which is now considered to afflict over 40% of the world's population with an increase in many developing nations (Welch and Graham, 2004; Guzmán *et al.*, 2014; Vrcek *et al.*, 2014). Micronutrient deficiency is common in developing countries, where staple cereals (wheat, maize or rice) provide most of the calories (Bouis *et al.*, 2011; Vignola *et al.*, 2016) since wheat and other cereal crops can suffer from micronutrient deficiencies, especially in Fe and Zn (Shi *et al.*, 2010; Vrcek *et al.*, 2014). Except for those nutritionally relevant minerals, there are also undesirable toxic metals in food such as cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), chromium (Cr) and aluminium (Al), among which, Cd, Pb and Hg have maximum residue levels (MRL) in food set by the European Commission.

Mineral including micro nutrient contents and heavy metals in cereal grain are the result of many factors including soil characteristic, agricultural practices, anthropogenic contamination, and genetic factors (Martínez-Ballesta *et al.*, 2010; Shi *et al.*, 2010; Zaccone *et al.*, 2010; Vrcek *et al.*, 2014). One of the aims of agricultural production of wheat is to increase the assimilation of micronutrients (Zn, Fe, Mn, Cu) and to reduce the absorption of toxic element (Cd, Hg, Pb) in grain (Shi *et al.*, 2010). One of purposes for the production of organic food is the reduction in the input of toxic substances. The meta-analysis based on 343 published papers indicated significantly lower Cd in organic crops compared with conventional ones (Baranski *et al.*, 2014), albeit that the values were lower than the MRL. Vrcek *et al.* (2014) also indicated that significantly higher levels of undesirable metals such as As and Cd were found in conventional compared with organic wheat flour. On the other hand, Worthington (2001b) surveyed the existing literature up to 2001 comparing the nutrient content of organic and conventional crops using statistical methods and showed that organic crops contained 21% more Fe, 29% more Mg, and 14% more P than conventional crops with this significant difference based on 41 studies and 1297 comparisons. However, whether organic wheat has more nutritionally relevant minerals and less toxic metals still needs more study to better understand the confounding effect of crop species, variety, environment condition/climate, fertilisation management as well as non-production system-specific management parameter (e.g. irrigation) on it, especially well-designed and controlled experiment study focusing on one specific crop and variety.

1.2.3 Mycotoxins

A review of the relevant literature discussing the mycotoxin content of cereal products under conventional and organic systems can be found in Chapter 2.1.1 "*background of meta-analysis for mycotoxin contamination in organic and conventional cereals*".

1.2.4 Pesticide residues

Pesticides are substances or mixtures of substances that are mainly used in agriculture or in public health protection programs in order to protect plants from pests, weeds or diseases, and humans from vector-borne diseases (Nicolopoulou-Stamati *et al.*, 2016). Based on target, pesticides include insecticides, fungicides, herbicides, rodenticides, and plant growth regulators. Based on chemical structure, pesticides can be classified into natural and synthetic (inorganic and organic). In addition, synthetic organic pesticides can be classified as: (a) organochlorine (DDT, Lindane, Endosulfan, Aldrin); (b) Organophosphates (Parathion, Malathion, Diaznon); (c) carbamates (Propoxur, Bendiocarb, Carbaryl) ; (d) pyrethroids (Deltamethrin, Cyfluthrin, Bifenthrin); and (e) others (Yadav and Devi, 2017). Based on toxicity criteria, pesticides can be classified as Ia (Extremely hazardous), Ib (Highly hazardous), II (Moderately hazardous), U (Unlikely to present an acute hazard) (Kim *et al.*, 2017).

The most common synthetic chemical pesticides/growth regulators used in wheat identified by this study included the insecticides Deltamethrin, Piperonyl butoxide, and Pirimiphos methyl and the plant growth regulator chlormequat.

Deltamethrin is an insecticide belonging to the synthetic pyrethroid family, which are man-made biochemicals similar to the pyrethrins, which are insecticidal phytochemicals naturally produced by chrysanthemum flowers. Deltamethrin is the active compound in a variety of broad-spectrum pesticide products and acts by disrupting the nervous system function of insects (Ortiz-Pérez *et al.*, 2005; Pohanish, 2017). Environmental and dietary exposure to synthetic pyrethroid-based pesticide products has been widely considered to have relatively low risks for human health (Barlow *et al.*, 2001). As a result synthetic pyrethroids are permitted for use in commercial insect spray and vapouriser products used for the domestic control of mosquitos and other insects (Barlow *et al.*, 2001). However, the main metabolites of pyrethroids have frequently been detected in urine samples from the general population, confirming widespread exposure of children and adults to one or more pyrethroids (Saillenfait *et al.*, 2015) and several recent epidemiological studies across the world have raised concerns about potentially adverse effects on the reproductive function of male adults (Ji *et al.*, 2011; Young *et al.*, 2013; Yoshinaga *et al.*, 2014; Jurewicz *et al.*, 2015). Therefore, further research is needed to clarify the possible risks associated with long-term environmental exposure to pyrethroids (Saillenfait *et al.*, 2015). The maximum residue level (MRL) for Deltamethrin set by the EU is 1 mg/kg (Table 1.1).

Piperonyl butoxide (PBO) is a synthetic, man-made pesticide synergist (Tozzi, 1999). It works by increasing the effectiveness of pesticides, and it is often combined with natural pyrethrins (pyrethrum) or synthetic pyrethroids in commercial pesticide products (Tozzi,

1999). Their mode of action is to inhibit the activity of enzymes produced by invertebrate pests that break down certain insecticides, thus increasing their efficacy (Farnham, 1999). The EU has currently not set an MRL for PBO, although there is concern about potential health risks linked to PBO-exposure. The chronic toxicity studies of piperonyl butoxide in rats found that PBO is a hepatocarcinogen to the rat (Takahashi *et al.*, 1994). The study by Grosman and Diel (2005) tested the effects and interaction between pyrethrins and PBO, and found the mammalian toxicity of pyrethroids can be ascribed to a general disturbance of cell membrane function in neuronal tissue as well as the immune apparatus on their own or enhanced by the mixtures. Natural pyrethrins are permitted in organic farming, however, when pyrethrins are mixed with PBO to make a pesticide more potent they are not allowed in organic farming systems.

Pirimiphos methyl is a post-harvest organophosphate (OP) insecticide used to control a variety of insects in stored grain products and seed such as corn, rice, wheat and sorghum. It was concluded that pirimiphos methyl (62.5 and 125 mg/kg) is detrimental to the reproductive potential of male rats (Ngoula *et al.*, 2007). The MRL set by the EU for pirimiphos methyl is 5 mg/kg and (Table 1.1). There is concern about negative human health impacts of both environmental and dietary exposure to OP-based pesticide and herbicide products including pirimiphos methyl (Eskenazi *et al.*, 1999; Ngoula *et al.*, 2007). Ngoula *et al.* (2007) found OP resulted in adverse effects on the reproductive function of adult male rats. A previous study by Eskenazi *et al.* (2007) investigated the relationship of prenatal and child OP urinary metabolite levels with children's neurodevelopment and found adverse associations of prenatal dialkylphosphate with mental development and pervasive developmental problems at 24 months of age.

Chlormequat (CCC) is a plant growth regulator belonging to the group of quaternary ammonium compounds. CCC is used in cereals (and most widely in common wheat) production to reduce stem length/longitudinal shoot growth (and thereby increase lodging resistance and improving harvest index and yield (EFSA, 2008; FAO, 2017). Long-term exposure to CCC was described to increase the risk of liver damage, tumours, and reduced reproductive and fetal health and fertility in animal models and/or humans (Sørensen and Danielsen, 2006; EFSA, 2010; LI *et al.*, 2011; Nisse *et al.*, 2015; Huang *et al.*, 2016).

Table 1.1 Maximum residue levels (MRLs) set by the EU and CODEX for deltamethrin, chlormequat, piperonyl butoxide and pirimiphos methyl

MRL set by the	Deltamethrin	Chlormequat	Piperonyl butoxide	Pirimiphos methyl
EU	1 mg/kg	4 mg/kg	NA	5 mg/kg
CODEX	2 mg/kg	3 mg/kg	30 mg/kg	7 mg/kg

Residues of pesticides can be found in a great variety of everyday foods. It should be noted that washing and peeling cannot completely remove the residues. In the majority of cases, the concentrations do not exceed the legislatively determined safe levels. However, these “safe limits” may underestimate the real health risk as in the case of simultaneous exposure to two or more chemical substances, which occurs in real-life conditions and may have synergistic effects (Nicolopoulou-Stamati *et al.*, 2016). There are to our knowledge, no comparative retail surveys in which (a) pesticide residues in common wheat and minor cereal products are compared and (b) confounding factors such as sample region (e.g. different countries), wheat production system (e.g. organic vs conventional) and grain processing method (e.g. white or wholemeal products) were considered. Therefore, there is an urgent need to investigate the potential physiological and health impact of reducing pesticide exposure via switching to organic food, wholegrain food, and minor cereal food consumption.

1.3 Irrigation and nutrition quality of cereals

Irrigation, together with nitrogen application, are the main agro-techniques for high yielding wheat production, and have a significant effect on grain quality (Ma *et al.*, 2015) . Water deficit is one of the principal factors restricting crop performance in arid and semiarid regions, due to irregular annual rainfall during the growing season (Wu *et al.*, 2017). Many studies have shown that appropriate increasing N fertiliser application rate can increase wheat yield and improve grain protein content. However, limited information regarding the managing irrigation of wheat for high phenolic phytochemical and minerals content is available (Saharkhiz Mj, 2012; Ma *et al.*, 2015). According to a previous study on sorghum, the deficit irrigation treatment significantly increased phenolic phytochemical content and antioxidant capacity in grain compared with the full irrigation treatment (Wu *et al.*, 2017). Pernice *et al.* (2010) planted tomato with three irrigation treatments and found that no irrigation and reduced irrigation could increase the flavonoid concentrations and antioxidant capacity of tomato fruits when compared with standard irrigation. However, results of some other studies found the opposite results (Gogorcena *et al.*, 1995; Zhang and Kirkham, 1996; Munné‐Bosch *et al.*, 2001). To our knowledge, there is no information and clear statement about the response in mineral content of wheat to water deficiency. Water deficiency increasing/decreasing phenolic phytochemical levels and mineral contents may depend on plant genotypes and confounding effect of species, fertilisation treatment and soil condition. To better understand nutrition performance of cereals under with/without irrigation, and to breed wheat varieties for arid and semiarid regions, well-designed and controlled experiments are required.

1.4 Spelt wheat

Spelt wheat (*Triticum spelta*), is one of the most ancient cereal species. It is a husked wheat species and was widely grown in Europe until the middle of the last century (Abdel-Aal and Hucl, 2005; Abdel-Aal and Rabalski, 2008), but its market share decreased rapidly during the post-war period of agricultural intensification (1950-2000) (Escarnot *et al.*, 2012). This was, at least partially, due to the additional processing required to remove the husks and the rapid advance in increasing yields of common wheat (*Triticum aestivum*) by breeding (e.g. introduction of semi-dwarfing genes) and of potatoes (Abdel-Aal and Hucl, 2005; Abdel-Aal and Rabalski, 2008).

More recently, the growing area and market share of spelt wheat has been increased again, and this coincided with the expansion of organic farming in Europe; Spelt wheat is a popular crop with organic farmers, due to its ability to grow under low input conditions and relatively good disease resistance and competitiveness against weeds. Also demand for spelt wheat and other minor cereals is particularly high in the organic food market, much of which is driven by consumers' perceptions that (a) minor cereals contain higher levels of beneficial mineral nutrients and phytochemicals and (b) organic farming practices will further improve the content of beneficial nutrients and decrease the content of undesirable compounds such as pesticides (Escarnot *et al.*, 2012). Similar to common wheat, spelt wheat is used to produce breakfast cereals, porridge, bread and other bakery products and different types of pasta, but the sensory characteristics of spelt wheat products are often different (Escarnot *et al.*, 2012).

Spelt wheat (*Triticum spelta*) is also known as *''Dinkel''* in German or *''Olyra''* in Greek has been cultivated since approximately 5000 BC, and was a major food crop for human nutrition in many regions of Europe from the Bronze age to medieval times. After the introduction of the potato into Europe in the 17th century it was increasingly replaced by the consumption of the this crop and from the beginning of the 20th century modern high yielding common wheat varieties which produce non-hulled grains became easier to process. However, it remained a staple food and minor cereal crop in parts of Central Europe and northern Spain, mainly because of its sensory characteristics (Abdel-Aal and Hucl, 2005; Abdel-Aal and Rabalski, 2008). For many years, cultivation of spelt wheat declined but recent interest in its nutritional characteristics and the expansion of organic production has led to a resurgence in its cultivation (Zieliński *et al.*, 2008a). Spelt wheat has been reported to have a relatively high resistance to environmental stress factors such as low soil fertility and fungal diseases. In addition, since the husks cover the seed, spelt wheat is thought to be relatively resistant against seed-borne and pre-emergence attack by soil-borne diseases and pests and less dependent on chemical seed treatment for good emergence rates. Spelt wheat varieties produce longer stems than modern short straw common wheat varieties, which is thought to

convey greater competitiveness against weeds, but also greater susceptibility to lodging, especially in intensive conventional production systems where, when high levels of mineral nitrogen fertilization are used, to increase yields and protein content. This explains why spelt wheat production has increased more rapidly in organic than conventional farming (Escarnot *et al.*, 2012).

European spelt wheat (*Triticum spelta*) was once considered as the progenitor of free-threshing *T. aestivum* species (McFadden and Sears 1946). However, later genetic studies showed that it arose through the introgression of cultivated emmer (*T. dicoccum*) wheat into free-threshing *T. aestivum* (Blatter *et al.*, 2002). *T. spelta* is a hexaploid wheat, which can easily be crossed with common wheat and as a result many "modern" high-yielding spelt which varieties grown widely in Central and Northern Europe are crosses between common and spelt wheat genotypes. Within the European organic sector there is a widespread perception that spelt wheat varieties originating from *T. spelta* x *T. aestivum* crosses produce grain with a lower nutritional value compared with the traditional, "pure" or "Ur-"spelt varieties. However, there are no scientifically sound studies in which the nutritional content and undesirable composition of "pure" *T. spelta* varieties has been compared with varieties originating from *T. spelta* x *T. aestivum* crosses.

In summary, there is very limited information about the concentration of phenolic phytochemicals, antioxidant capacity, nutritional minerals and phenolic profile as well as undesirable composition such as pesticide residues and mycotoxin contamination (a) between common wheat and spelt wheat, as well as (b) between ancient spelt wheat and modern spelt wheat, especially under organic vs conventional farming management and with/without irrigation.

1.5 Whole grain

Cereal whole grains consist of three main parts: endosperm, bran, and germ (Figure 1.1). Inclusion of whole grains in the diet is more and more recommended in dietary guidance around the world because there is strong scientific evidence for health benefits associated with increased consumption of whole grain (Jones and Engleson, 2010). Whole grains are linked to reduced risk of obesity or weight gain; reduced risk of cardiovascular disease; improving gut health and decreased risk of cancers of the upper gut; perhaps reduced risk of colorectal cancer; and lower mortality rates (Jones and Engleson, 2010; Borneo and Len, 2012). Some studies have been carried out to determine whether their health benefits are due to the synergy of whole grain components, individual whole grain components, or the fact that whole grain consumers make many of the recommended beneficial diet and lifestyle choices

(Jones and Engleson, 2010). Whole grains have always been recognized for their contribution of traditional nutrients, vitamins B, minerals, and dietary fibre, to the diet. More recently, whole grains have been shown to be a good source of antioxidants (Jones and Engleson, 2010). Since in Europe and worldwide most cereal products, like white bread and pasta, are based on kernels or flour after removal of bran and germ, the two outer parts containing most of the dietary fibre and other bioactive components (van der Kamp *et al.*, 2014), comparing the differences nutritional composition and contamination between whole grain and white flour enable us to understand the possible nutrition advantages of whole grain and associated health benefits.

On the other hand, whole grain/wholemeal products may also have a higher concentration of undesirable components such as pesticides and mycotoxins, which could also be a potential risk for health. To our knowledge, there are no publications comparing mycotoxin contamination and pesticides residues between whole-grain and white flour. Therefore, studies focusing on the undesirable components in whole grain and whole-grain products are required to better understand the advantage and ways to avoid the disadvantages of whole grain consumption to provide more comprehensive diet suggestion for customers.

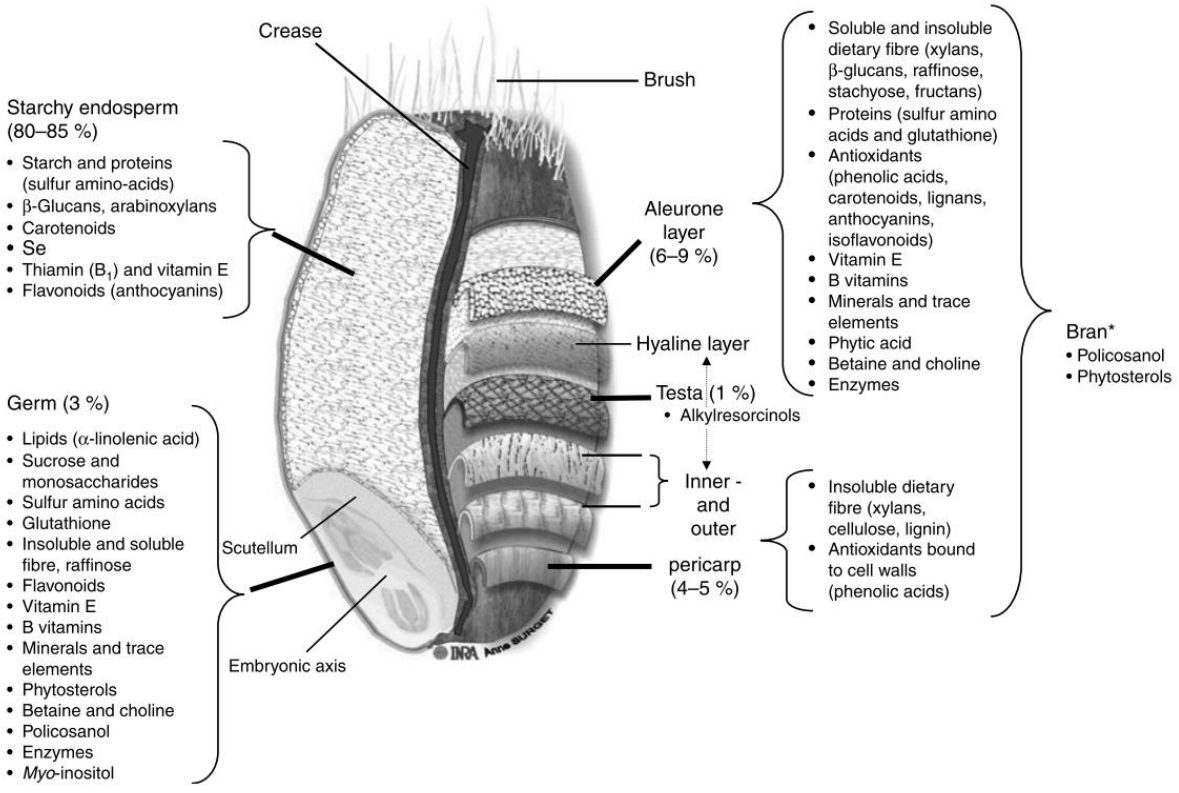


Figure 1.1 The three wheat fractions (bran, germ and endosperm) with their main bioactive compounds (Fardet, 2010).

1.6 Aims and objectives of the work

The **overall aim** of the project was therefore to obtain a more accurate estimate of the nutritional differences and undesirable components' differences between organic vs conventional cereals (mainly wheat), while accounting for the confounding effects of wheat species (common vs spelt wheat), flour type (white vs wholegrain), country (UK vs Germany) and the specific agronomic practices and climate condition potentially affecting the yield and quality of wheat grain. This was done by:

- A systematic literature review (meta-analysis) describing (a) effects of organic and conventional agronomic practices and (b) correlation between farming systems and climate types, countries and cereal types on the mycotoxin contamination of cereals;
- Quantification of the effects of (and interactions between) farming system (conventional vs organic), country (UK and Germany), species (*T. aestivum* vs *T. spelta*) and flour types (wholemeal vs white) on nutrition related component contents and undesirable composition including pesticides and mycotoxins (deoxynivalenol, zearalenone, T-2 toxin and ochratoxin A) concentration in bought supermarket flours;
- Quantification of the effects of (and interactions between) contrasting spelt genotypes *(T. spelta*), supplementary irrigation and fertiliser input types on performance of spelt wheat in a semi-arid region of the Mediterranean.

1.7 Links between the three main chapters

To illustrate the structure of the thesis, links between the main chapters 2, 3 and 4, respectively for meta-analysis, shopping basket survey and Crete field experiment are presented in figure 1.2. The most important shopping basket survey chapter (Chapter 3), comprehensively investigated the nutritional quality (phenolic phytochemicals and macro and micro minerals) and undesirable constituents (mycotoxin, pesticide and heavy metals) of common and spelt wheat to understand the effects of farming system (organic vs conventional), flour type (white vs wholemeal) and wheat species (common vs spelt). Among those parameters, mycotoxin contamination in food is becoming a global food safety issue possibly as a consequence of climate change. However, only one meta-analysis has been carried out on this topic and it only was based on a small number of publications. To make best use of the published papers and data related to mycotoxin content of cereals and to verify the finding of the shopping basket study in this thesis, a meta-analysis (Chapter 2) was carried out mainly comparing the mycotoxin contamination in organic versus conventional cereals. In addition, to explore what agronomic practices affecting the nutritional quality of the minor cereal spelt wheat, the field experiment (Chapter 4) was carried out to identify the effects of fertility and irrigation treatment and spelt variety on nutritional quality of spelt grain.

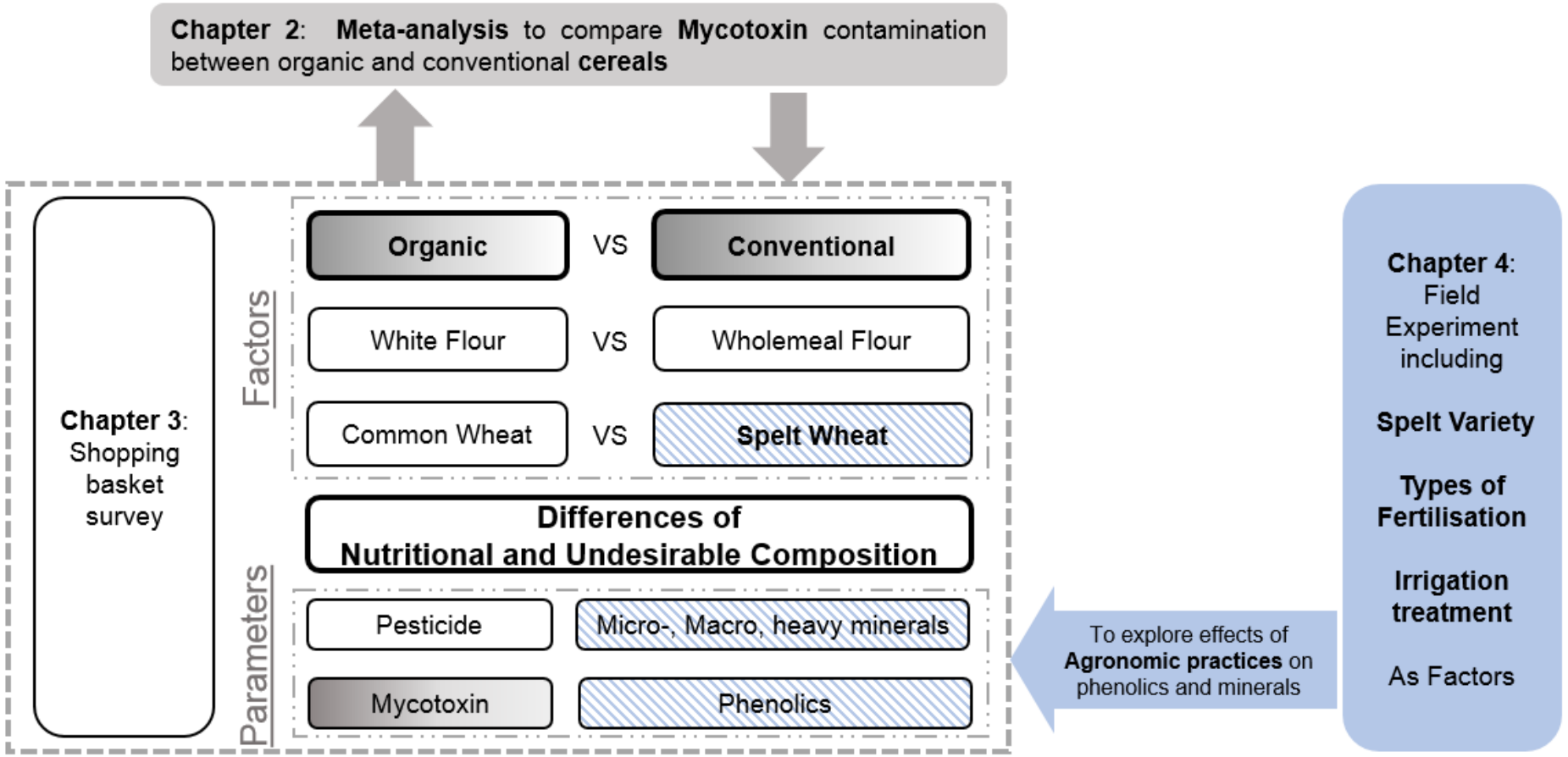


Figure 1. 2 Flow diagram indicating the links between the three main chapters

Chapter 2 Mycotoxin content in organically versus conventionally cultivated crops: a systematic literature review and meta-analysis

2.1 Background

2.1.1 The problem

Mycotoxins are secondary metabolites synthesized by specific fungi (Köpke *et al.*, 2007). When given favourable conditions, fungi can infect almost every agricultural product (e.g. cereals, nuts, fruits, etc.) during plant growth and/or after harvest (Dall *et al.*, 2015). Mycotoxins are undesirable ingredients of food and feeds, since they can cause harm to human and livestock health through dietary and feed exposure. The effects of mycotoxins could be acute or chronic. For example, deoxynivalenol (DON) causes vomiting, and the symptoms, which can be very severe, appear very quickly. In contract, ochratoxin A (OTA), aflatoxins and other mycotoxins have chronic or cumulative effects on health, including carcinogenicity, mutagenic and immunosuppressive effects (Bryla *et al.*, 2016; Ferrigo *et al.*, 2016). Another impact of the presence of mycotoxins is that they cause significant economic losses (Barug *et al.*, 2006). The United States Food and Drug Administration (FDA) estimated, using computer modelling, that the impact of mycotoxins in the U. S. to the economy from crop losses (corn, wheat and peanut) ranged from $418 million to $1.66 billion annually (CAST, 2003).

Although over 300 fungal secondary metabolites are known, it is widely agreed that, in agriculture, there are five most important naturally occurring mycotoxins in human foods and animal feeds: aflatoxins, fumonisins, OTA, DON, and zearalenone (ZEA). These toxins are produced by just a few species from the genera *Asperigillus, Penicillium, Fusarium, and Claviceps* (Table 2.1) (Barug *et al.*, 2006; Pitt *et al.*, 2012). The three main fungal groups responsible for mycotoxin contamination in cereal grains are (a) *Fusarium* species, which produce trichothecenes, zearalenones and fumonisins in grains (Ferrigo *et al.*, 2016), (b) common mould fungi (*Penicillium* and *Aspergillus* spp.), which produce aflatoxins and ochratoxin A (OTA); and (c) *Claviceps* spp. (especially *C. purpurea*) which produce the ergot alkaloids/mycotoxins (Barug *et al.*, 2006; Pitt *et al.*, 2012).

Table 2.1 The five agriculturally important mycotoxins, their main producing fungi and the main products affected

Mycotoxin	Main producing fungi	Main crops affected
Aflatoxins	*Aspergillus flavus, Aspergillus parasiticus*	Maize, nuts, rice, tree nuts
Ochratoxin A (OTA)	*Aspergillus ochraceus, Penicillium verrucosum,*	Maize
Deoxynivalenol (DON)	*Fusarium graminearum, Fusarium culmorum*	Cereals, wheat
Zearalenone (ZEA)	*Fusarium graminearum, Fusarium culmorum,*	Maize, wheat
Fumonisins	*Fusarium verticillioides Fusarium proliferatum*	Maize

2.1.2 Fusarium species and their toxins

Fusarium species, which can be isolated from soil and plant materials, are widely distributed both in temperate and tropical regions of the globe. It is one of the most economically important genera of phytopathogenic fungi, causing diseases in the host plant such as *Fusarium* Head Blight (FHB), and leading to a severe reduction in yield and quality (Munkvold, 2003; Nganje *et al.*, 2004; Backhouse, 2014). *Fusarium* infection affects cereals such as wheat, barley, oats and ear rot of maize when in the field, and could produce three of the five most important naturally occurring mycotoxins in human food and animals feed, including trichothecenes, zearalenones and fumonisins (Ferrigo *et al.*, 2016).

Trichothecenes produced by *Fusarium spp.* are widespread in all cereal-growing areas of the world (Shank *et al.*, 2011). Characterised by the presence of different functional groups, trichothecenes are divided into two groups: A and B (Shank *et al.*, 2011; Ferrigo *et al.*, 2016). The A group includes T-2 toxin and HT-2 toxin, and DON and nivalenol (NIV) are in group B. DON has the potential to cause chronic effects such as reduced growth and anorexia, as well as acute intoxication leading to vomiting (emesis), immunotoxic effects and changes in brain neurochemicals (Authority, 2004). The T-2 and HT-2 toxins are also of considerable concern for human and animal health being potent inducers of oxidative stress and inhibitors of DNA, RNA and protein synthesis and mitochondrial functions (Yang *et al.*, 2016).

ZEA contamination often co-occurs with DON. ZEA is of major interest because, despite its low acute toxicity, it has been shown to be hepatotoxic, immunotoxic, and carcinogenic to a number of mammalian species (Cortinovis *et al.*, 2013). Moreover, ZEA and some of its metabolites have been shown to competitively bind to estrogen receptors in a number of

different species and are responsible for hyper-estrogenism and infertility in livestock (Cortinovis *et al.*, 2013; Ferrigo *et al.*, 2016).

There are up to 13 *Fusarium* species which have been shown to produce fumonisins, which are correlated with oesophageal cancer in humans (Sydenham *et al.*, 1991). It was shown that more frequent incidences of oesophageal cancer in regions of the Transkei (South Africa), China and northeast Italy were correlated with the occurrence of fumonisin B_1 (Peraica *et al.*, 1999b).

2.1.3 Common mould fungi (Penicillium and Aspergillus spp.) and their toxins

OTA is a mycotoxin produced by some species of *Penicillium* and *Aspergillu*s (Köpke *et al.*, 2007). Two major OTA producing Penicillium species and currently recognized: *Penicillium nordicum*, which is mainly found on meat and cheese, and *Penicillium verrucosum*, which is found to contaminate grain. The Aspergillus species are also known to produce OTA including *Aspergillus niger*, which is thought to be responsible for OTA production in wine and *Aspergillus ochraceus*, the main OTA producer in coffee and cocoa. OTA is nephrotoxic to all animals tested and the causal agent of mycotoxin porcine nephropathy (Berndt *et al.*, 1980; Köpke *et al.*, 2007). OTA was previously associated with the human renal disorder, Balcan Endemic Nephropathy, and tumours of the urinary tract (Pfohl-Leszkowicz *et al.*, 2002; Köpke *et al.*, 2007). Recently, another endemic kidney disease was linked to OTA-contaminated food and it has also been linked with testicular cancer (Schwartz, 2002; Köpke *et al.*, 2007).

Aflatoxins are mainly produced by *Aspergillus flavus* and *A. parasiticus.* Aflatoxins are the most potent natural chemicals to cause liver carcinogens known. The combination of aflatoxin with hepatitis B or C, prevalent in China and sub-Saharan Africa, is synergistic, raising more than tenfold the risk of liver cancer compared with either exposure alone (Miller and Marasas, 2002; Barug *et al.*, 2006) . Aflatoxins are also associated with stunting in children and possibly immune system disorders (Turner *et al.*, 2003; Barug *et al.*, 2006; Wild *et al.*, 2015). Aflatoxins mainly cause cancer of the liver, kidneys and bile duct. What is more, aflatoxins have the highest toxicity of all known mycotoxin (Köpke *et al.*, 2007).

2.1.4 Claviceps and other fungi-producing ergot alkaloids

Ergot alkaloids are produced by *Claviceps purpurea,* which has contaminated rye flour for centuries and is still a problem in many areas of the world. Poisoning from *C. purpurea* dates back to antiquity. Several epidemics have been reported since the Middle Ages that were linked to the ingestion of mouldy rye bread. C. *purpurea* is a species complex that, beside rye, can infect wheat, barley, triticale and more than 600 grasses, especially under cool and rainy weather conditions. Thus, infection can also originate from grasses growing off-site or as weeds in the stand (Agrios, 1997; Köpke *et al.*, 2007).

2.1.5 Maximum limit for mycotoxin in cereals

Cereals are the main source of mycotoxins intakes by humans in both developed and developing countries (Leblanc *et al.*, 2005; Nleya *et al.*, 2018). Mycotoxins contamination of cereals and other crops has only relatively recently been recognised as an important food safety issue (Wild and Gong, 2010; Bryła *et al.*, 2016). Concern is based on epidemiological evidence for harmful effects of mycotoxins on both human and animal health, which was described previously (Wild and Gong, 2009; Streit *et al.*, 2012; Nleya *et al.*, 2018; Schaarschmidt and Fauhl‐Hassek, 2018). To minimise human exposure to harmful concentrations of mycotoxins the European Union has set legal limits for mycotoxin contamination in cereals and cereal products (European Commission, 2006a). (European Commission, 2006a). Currently maximum mycotoxin contamination levels set by the EU include (a) 750 and 500 µg DON per kg for unprocessed cereals intended for human consumption and bread respectively, (b) 75 µg ZEA per kg of cereals intended for direct human consumption, (c) 200-1000 µg total fumonisins (B1 + B2) for maize and maize based foods for direct human consumption (depending on the type of product), (d) 5 and 3 µg OTA per kg of unprocessed and processed cereals respectively, (e) 0.5 µg OTA per kg of cereal based foods and baby foods for infants and young children and (f) 2 and 4 µg/kg respectively for AF-B1 and total aflatoxins (B1, B2, G1 and G2) in cereal grains/products (European Commission, 2006a). More details were shown in the table 2.2. The EC has also made a recommendation for maximum contamination levels for the sum of T-2 and HT-2 mycotoxin (50 µg/kg) (European Commission, 2006b), but there is currently no legal EU-limit for H-2 and HT-2 Fusariotoxins contamination (European Commission, 2006a). However, H-2 and HT-2 were linked to negative health impacts at much lower concentrations than DON and other *Fusarium* mycotoxins (Adhikari *et al.*, 2017).

Table 2.2 US and EU limits on food level for mycotoxins

Mycotoxin	US FDA	EU (EC 2006)	For wheat flour (EU)
		ppb	
Aflatoxins	Total: 20	B_1: 0.1-12 Total: 4-15	B_1:2 Total: 4
Deoxynivalenol	1000	200-1750	750
Fumonisins	2000-4000	B_1&B_2: 200-4000	Not Set
HT-2/T-2	Not Set	15-1000	50
NIV	Not Set	Not Set	Not Set
Ochratoxin A	Not Set	0.5-80	3
Zearalenone	Not Set	20-400	75

2.1.6 Why agronomic systems may affect mycotoxin levels in cereals

Fungicides are widely used in conventional cereal production to control foliar diseases (including *Fusarium* head blight), but are prohibited under organic farming standards. It has therefore been argued that organic cereal crops are at a higher risk from fungal diseases and mycotoxin contamination (Gomiero, 2017). However, studies into the effect of fungicides application on *Fusarium* head blight severity *Fusarium* grain infection and mycotoxin levels often showed variable and/or contradictory results (Magan *et al.*, 2002; Heier *et al.*, 2005). There is also evidence that the use of fungicides may increase mycotoxin production due to stress imposed on the fungal pathogen (Köpke *et al.*, 2007).

The use of diverse-rotations is known to reduce *Fusarium* disease pressure and mycotoxin risk by (a) minimizing the build-up of soil/crop residues and breaking the life cycle of a fungal pathogens (Pirgozliev *et al.*, 2003). In contrast, cereal monocultures and especially growing cereals such as wheat after maize substantially increases the risk of *Fusarium* head blight and mycotoxin contamination (Krebs *et al.*, 2000; Köpke *et al.*, 2007).

The susceptibility to *Fusarium* and *Claviceps purpurea* infection and associated mycotoxin risks differs considerably between cereal species. For example, maize and wheat are considered to be more at risk from *Fusarium* infection/mycotoxins than rye, barley and oats; while rye is most at risk from *C. purpurea* infection and ergot contamination (Foroud and Eudes, 2009). There are also substantial differences in susceptibility between varieties of the same cereal species. For example, longer straw varieties are usually less susceptible to *Fusarium* infection/mycotoxin contamination (Köpke *et al.*, 2007; Foroud and Eudes, 2009), unless lodging occurs (Nakajima *et al.*, 2008; Konvalina *et al.*, 2016).

Other agronomic management factors linked to an increased risk of fungal infection and mycotoxin contamination include (a) high inputs nitrogen fertiliser (Bernhoft *et al.*, 2012; Supronienė *et al.*, 2012), (b) use of minimum or no tillage systems (Dill-Macky and Jones, 2000; Oldenburg *et al.*, 2007; Supronienė *et al.*, 2012), (c) insufficient drying of cereal before storage (Magan and Aldred, 2005; Magan and Aldred, 2007; Magan *et al.*, 2010) and (d) use of farm saved seed, especially where appropriate storage and seed cleaning (e.g. to remove ergot scerotia) facilities are not available (Schumann & Uppala 2000; AHDB 2018). However, it is not possible to extrapolate the relative risk of mycotoxin contamination associated with organic and conventional cereal production systems from the results of these studies. This is mainly, because some agronomic practices linked to an increased mycotoxin risk are more commonly used in organic systems (e.g. use of farm saved seed), while others (e.g. high nitrogen fertiliser inputs, use of minimum/no tillage systems) are more prevalent in conventional farming systems (Veeresh and Veeresh, 2006).

2.1.7 Why it is important to do the review

Over the last 30 years more than 70 farm and retail surveys, and field experimental studies have been carried out to compare mycotoxin prevalence and/or concentrations in organic and conventional cereals gains and cereal products (e.g breakfast cereals, pasta, bread). However, the available quantitative literature reviews came to inconsistent and contrasting conclusions as to the relative risk of mycotoxin contamination in organic and conventional cereals and other foods (Trewavas, 2004; Magkos *et al.*, 2006; Lairon, 2010a; Gourama, 2015; Brodal *et al.*, 2016b). These reviews can be criticises for selective use of the available data and have not contributed to reducing the considerable uncertainty about the relative risk of mycotoxin exposure from organic and conventional cereals.

There has been only one study in which a systematic review/meta-analysis approach was used to compare mycotoxin risks in organic cereals/cereal products (Smith-Spangler *et al.*, 2012b). This study reported a higher risk for OTA contamination in organically grown rice, but not wheat, but lower levels of DON in organic wheat. However, DON and OTA data from only 8 and 9 studies respectively were used in their analyses.

Therefore, it is important to repeat a meta-analysis using data from the growing number of recent studies, and employ a more advanced methodology, in order to gain a better insight into the role of agricultural management and possible specific agronomic and pedo-climatic factors influencing the mycotoxin contamination of crops.

2.2 Objectives

The main objective of the systematic review/meta-analyses reported here was therefore to identify and quantify difference in mycotoxin prevalence and concentrations between cereals and cereal-based products produced in conventional and organic farming systems.

Additional objectives were to identify/quantify ‘confounding’ effects of (a) cereal species, (b) country/climatic zone, (c) study type (farm and shopping basket surveys, and experimental studies), (d) time (years in which studies were carried out) and (e) mycotoxin analysis method. In addition, we carried out a range of sensitivity studies to identify the impact of data management (e.g. inclusion criteria, meta-analysis method) on the overall results of the meta-analysis?

2.3 Methodology

Methodology for the review was based on previously published protocols by Wang *et al.* (2018).

2.3.1 Criteria for including and excluding studies

Types of study designs

Data included in this review consisted of published results for samples of cereal grains, milled flour or cereal-based foods from grains cultivated under organic or conventional agriculture management systems. Based on the sample collecting protocol and location described in comparative studies used, the review included three types: comparisons of matched farms (CF), shopping basket studies (SBS) or controlled field experiment (EX). Studies with matched farms comparisons, also called farm surveys, included samples collected from organic and conventional farms in the same country or regions, and the number of farms was considered as sample size in the meta-analysis. Shopping basket studies or retail product surveys used products labelled as organic or not- labelled as organic (conventional) from retail outlets in given areas. Organic labels were compliant with European Union (EU), United Stated Department of Agriculture (USDA) or other national government agency certification schemes. Comparisons in controlled experiments were based on analyses of grains from crops which were grown in field experiments with a randomised block design, and the sample size was the number of replicate plots used in the experiment. The review included data from all possible published studies, as well as the results from unpublished experiments obtained directly from research groups.

Types of participants

The study population were all cereals and cereal-based products grown and produced under organic and conventional production systems including common wheat, barley, oat, spelt, rye, rice, emmer, buckwheat, sorghum, millet, triticale, fonio, and quinoa. Maize (corn) as it is used mainly for feed production was excluded in this study.

Types of interventions

Only studies directly comparing mycotoxin contamination between grain, flour and cereal-based foods of organic and conventional origin were included in this review. As conventional, the farming systems commonly using application of mineral fertilisers and pesticides, or the products made from crops grown in this way were recognised. As organic, the farming systems defined as certified to organic farming standards or using organic methods, or experimental plots under organic management, as well as products from cereals or cereal-based foods labelled as organic were considered.

In several studies terms other than “conventional” or “organic” were used to describe non-organic and/or organic management practices/protocols. Therefore, management systems named ‘integrated’, ‘low input’, ‘extensive’, and protocols which according to the authors’ description involved the use of mineral fertilizers and/or pesticides, were treated as conventional. Farming systems described or certified as called ‘biodynamic’, ‘biological’ or ‘ecological’, that followed the “organic” principles and omitted the use of synthetic chemical mineral N and P fertilisers and pesticides in the production protocol, and/or described that organic fertilisation and crop protection regimes were used, were deemed as organic.

Some studies, compared more than two production systems which could be treated as organic and/or conventional. This might include different types and levels of nitrogen input, presence or absence of fungicides, or other treatment variation. However, only the organic versus conventional (non-organic) system identified by the author of each study as closest to the typical, contemporary organic/conventional farming system was included in the standard meta-analysis, as recommended previously (Brandt *et al.*, 2013; Barański *et al.*, 2014; Wang *et al.*, 2018).

Types of outcome measures

All mycotoxin data found in papers were included in this review. Apart of measures of concentration in the given unit per weight (µg/kg, ppb, ng/g), data on the frequency of detection (proportion of samples testing positive for the presence of a given mycotoxin) were recorded and considered in the meta-analyses.

2.3.2 Search strategy

The literature search strategy was based on previously published protocols by Wang *et al.* (2018) with specific adjustments for this review topic.

Papers relevant for the review were identified by the initial search in three online database including Web of Science, Scopus and EBSCO. The research phrases contained four groups of terms combined with Boolean logic (“OR”, “AND”) and with asterisk truncation (*) in order to find all contrasting interventions and participants for selected outcome:

- (Organic* OR ecologic* OR biodynamic*) AND
- (Conventional* OR integrated) AND
- (wheat OR barley OR oat OR spelt OR rye OR rice OR emmer OR buckwheat OR sorghum OR millet OR triticale OR fonio OR quinoa OR cereal*) AND
- (deoxynivalenol OR aflatoxin OR beauvericin OR diacetoxyscripenol OR enniatins OR fumonisin OR fusarenon X OR HT-2 OR T-2 OR monoacetoxyscirpenol OR moniliformin OR neosolaniol OR nivalenol OR ochratoxin OR zearalenone OR mycotoxin*)

The search was restricted to the period between January 1992 and December 2017. This covered studies which conducted from the year when legally binding organic farming regulations were first introduced in the European Union to the most recent studies.

Studies published before 1992 were obtained from qualitative review papers and also included in the meta-analyses.

Papers in all languages were included and the translation of papers, that were published in languages other than English, were carried out by member of the team or external scientific collaborators.

2.3.3 Details of study coding categories

Screening and data extraction

The first screening stage of papers identified in the on-line search involved the evaluation of the titles and abstracts. All papers that mention comparisons of mycotoxin level in cereal and cereal-based food in organic and conventional foods or farming systems were recorded into the database. Publications obtained from each online database were merged after the first stage of screening, and any duplicates were removed. In the second screening stage, the full text of the papers was read to identify suitable data-sets. If the full texts of publications could not be found or obtained, re-prints were requested directly from the authors. All available publications were independently read evaluated for suitable data-sets by two reviewers, to minimise the chances of suitable data being missed and to confirm the eligibility of data included in analyses. In addition to data on the frequency of detection and concentrations of mycotoxins a wide range of background data (e.g. years, country/regions and/or climatic zones in which studies were carried out, sponsors of studies, details of agronomic practice, and assessment and analytical methods used) were also extracted and recorded in the database, and/or used in analyses (Appendix 2.1).

Data reported as numerical values in the text or tables was copied directly into the database. Data published in graphical form was enlarged, printed, measured (using a ruler) and then enter into the database. All discrepancies and disagreements were discussed and resolved by the whole reviewers group. During data extraction, the list of references in publications was checked as well to find more eligible publications. In additional, we contact the authors of all collected papers with requests for information on other publications or unpublished results. A summary of the data search and selection process is presented in a flow diagram, which also includes information on the number of papers found and excluded, reasons for exclusion and the final number of papers included in data extraction and the standard weighted and unweighted meta-analyses.

Climate data

Climate is considered as a key factor affecting the persistence, infection and development of fungal species in plant tissues and also mycotoxin production and contamination in cereal grains, and climatic data were therefore extracted from publications. However, not all of the included papers provided information on climatic conditions or considered climate as a factor in the statistical evaluation, or provided only a short insufficient communication. In this review, we therefore did not use climate information provided in individual papers in multi-level model meta-analyses. Instead unified climate and weather information according to the Koppen-Geiger climate classification scheme (Peel *et al.*, 2007) was used for the geographic location of cultivation reported in CF and EX studies and the country of origin in SBS-studies.

Dealing with missing data, 'not detected' and 'zero' values

In this review the meta-analytical evaluation included standard protocol in which values of detected contamination were used (see also below), and additional protocols in which the summary were calculated for all samples including 'not detected' and 'zero' values. Whenever studies with missing concentration data were discovered, for which the toxin presence was detected, the authors were contacted in an attempt to obtain all missing values. If not successful, other available data in the paper was used to calculate the effect size in order to perform calculations (Lajeunesse, 2013). For example 'zero', 'not quantified' or 'not detected' values were replaced with the half of the limit of detection (LOD) or the half of the limit of quantification (LOQ) provided in the study. In addition, when both mean and median value were missing in studies, and there was only contamination percentage. In this situation, if the contamination percentage in total samples was less than 50%, half of the LOD/LOQ was regarded as the median for calculation in the meta-analysis. If the contamination percentage was more than 50%, only the value "percentage" was recorded, the mean was left blank. For positive samples with missing variation details (SE or SD), and for which the range (minimum and maximum) was known, when the percentage was less than 100%, the minimum was half of the LOD/LOQ. Furthermore, the method using rpois() function in R was employed for imputation of the missing values of standard deviation. Studies for which calculations were possible were not excluded from the meta-analysis.

For some papers only reporting means of positive samples, if details of total samples' number, positive samples' number and LOD/LOQ were available in papers, the following equation was be used to do the transformation.

$$means\ for\ all\ samples = \frac{positive\ mean * No.of\ postive\ samples + (total\ No.of\ samples - No.\ of\ positve\ samples) * 0.5 * LOD}{Total\ No.of\ samples}$$

Dealing with other unclear information

For studies which did not report the year in which experiments of surveys were carried out we assumed that the study was carried out two years prior to the year of publication (Champeil *et al.*, 2004; Twaruzek *et al.*, 2013; Blajet-Kosicka *et al.*, 2014).

When studies reported data for replicate samples or raw data for the same system, crop, year and country, replicate data for each combination of crop, year and country of origin were averaged and SEs calculated. For replicate samples, in which no mycotoxins were detected (concentrations < LOD) half the LOD (as described the paper) was used in the calculation of means.

Assessment of risk of bias in included studies

Studies included in the review were critically appraised and evaluated for potential source of bias associated with the study design, analytical methods, selective outcome reporting and conflicts of interest. Assessment had a form of statements with three optional answers: (1) “YES” when the statement reflected the content of the paper; (2) “NO” when there was no information in the paper described by the statement; or (3) “Unclear” when information provided did not reflect the statement. The last point on the checklist was the final rating of the overall methodological quality of the study. None of the studies was excluded from the standard meta-analysis based on these quality assessments; however, results of the publication quality assessments were taken into account during the evidence synthesis as part of the GRADE (Grading of Recommendations, Assessment, Development and Evaluation) system report, and were used to exclude certain studies in the sensitivity analyses .

2.3.4 Data synthesis

Characteristics and findings of each study included in the Literature Review were presented as descriptive results. Both meta-analytical protocols, weighted and unweighted, were carried out using the “metafor” package in the R statistical environment (Wang *et al.*, 2018). Data on concentrations and the frequency of positive samples were analysed separately for each mycotoxin.

2.3.5 Weighted meta-analysis

The most frequently reported values for mycotoxin contamination in cereals were (a) the mean concentration in all samples (estimated by using the measured values for positive samples and half the quantification limit of the analytic test as the value for negative samples) and (b) the proportion of samples testing positive for mycotoxin-contamination. The two "standard" weighted meta-analysis that were carried out, were therefore based on two effect sizes: the mean differences of mycotoxin concentrations (based on reported estimates based on using the measured values for positive samples and half the detection limit of the analytic test as the value for negative samples) and the odds ratio for comparing the proportions of organic and conventional samples in which mycotoxins were detected. For both meta-analysis methods the corresponding sampling variance (confidence intervals) were also calculated in R using the "metafor" package.

The outcomes reported in included studies were on the same meaningful scale, thus the meta-analysis could be performed directly on the raw difference of means (MD) (Borenstein, 2009). The MD was calculated as

$$MD = \bar{X}_o - \bar{X}_c$$

Where $\bar{X}_o$ was the concentration mean of organic samples, and $\bar{X}_c$ the concentration mean of conventional cereals.

The variance of MD was calculated as follows, with the S_1 and S_2 being the sample standard deviations of the two groups, and n_1 and n_2 being the sample sizes in the two groups:

$$V_{MD} = \frac{n_1 + n_2}{n_1 n_2} S^2_{pooled}$$

where

$$S_{pooled} = \sqrt{\frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2}}$$

A positive MD value indicated that the mean concentration of the observed mycotoxin was higher in the samples of organic cereals, while a negative MD indicated that the mean concentrations was higher in the samples of conventional cereals.

For studies reporting data on the frequency of occurrence of detectable mycotoxin concentrations in cereals, the **odds ratio** (OR) was used. It is an effect size based on binary data (Borenstein *et al.*, 2011) (Table 2.3).

Table 2.3 Nomenclature for 2×2 table of outcome by treatment

	Contaminated	**Non-contaminated**
Organic	a_i	b_i
Conventional	c_i	d_i

The OR was calculated on the logarithmic scale as:

$$Odds\ Ratio = \frac{a_i \times d_i}{b_i \times c_i}$$

Where a_i is the number of contaminated samples in organic crops, b_i is the number of non-contaminated samples in organic crops, c_i is a number of contaminated samples in conventional crops, and d_i is a number of non-contaminated samples in conventional crops.

A positive value of the odds ratio meant that the frequency of occurrence of mycotoxin was higher in organic samples, and a negative value of the odds ratio meant that the frequency of occurrence of mycotoxins was lower in organic samples.

Tests of homogeneity (Q statistics and I^2 statistics) were carried out on all the summary effect sizes. Homogeneity was indicated if I^2 was less than 25% and the P value for the Q statistics was greater than 0.01.

Potential effects of **moderators** on mycotoxin contamination in cereals from conventional and organic production systems, such as cereal species, climate, country, and study type were explored using mixed-effect models and subgroup analyses.

Potential **publication bias** was assessed by inspection of funnel plots and using the Egger's regression test for funnel plot asymmetry. When the publication bias was evident, the Trim and Fill method was used to calculate approximate number of "missing" studies (Wang *et al.*, 2018).

The overall **strength of evidence** derived from the meta-analysis was explored using an adaptation of the Grading of Recommendations, Assessments, Development and Evaluation (GRADE) framework, which includes information about risk of bias for each study, as well as inconsistency, indirectness and imprecision of the results, and publication bias.

Sensitivity analyses were conducted to explore how data management and inclusion criteria affected the overall results of the meta-analyses. The potential impacts were assessed of (a) excluding all publications deemed to be of poor and acceptable quality and (b) using averaged

mycotoxin concentration and proportion by years when studies were carried more than one year.

2.3.6 Identifying changes in mycotoxin concentration over time

In addition, we carried out first order regression analysis to study trends of DON and OTA (Figure 2.2), ZEA, total fumonisins, total T-2/HT-2, total aflatoxins (Appendix 2.4) mycotoxin contamination levels over time. To identify potential changes in mycotoxin loads of cereals over time, mean concentrations of the different mycotoxins in organic and conventional cereals/cereal reported before 2004 (the year when the legal EU-standards for crops came into effect) and up to two successive 5-year periods after 2004 (2004 to 2009, and 2010 to 2015) were calculated. These means were then used to calculate 1st order regressions through the mean concentrations for both organic and conventional samples for the different time periods (Figures 2.2 and Appendix 2.4).

2.3.7 Unweighted meta-analysis

In order to include mean values extracted from publications in which measures of variability and/or sample size were not provided, unweighted meta-analyses were also carried out. The effect size was calculated as an In-transformed ratio of the concentration of mycotoxin in an organic sample to the concentration of mycotoxin in a conventional sample ($\bar{X}_o/\bar{X}_c$) , and was expressed as a percentage. The significance of difference between samples was evaluated comparing the arithmetic average of the result using a resampling method. *P*-values were derived from Fisher's one-sample randomisation test and a $P<0.05$ was considered statistically significant (Wang *et al.*, 2018).

$$Ratio = \frac{\bar{X}_o}{\bar{X}_c} \times 100\%$$

2.4 Results

A total of 398 publications were identified in the initial literature search, 249 of which were excluded after reviewing the title and abstract. The full texts of the remaining 149 papers were read, and 76 of which did not report suitable data were rejected. Overall, 73 peer-reviewed publications fulfilled the criteria of the meta-analysis defined in the protocol (Appendix 2.3). Flow diagrams of the search and selection process are shown in Figure 2.1.

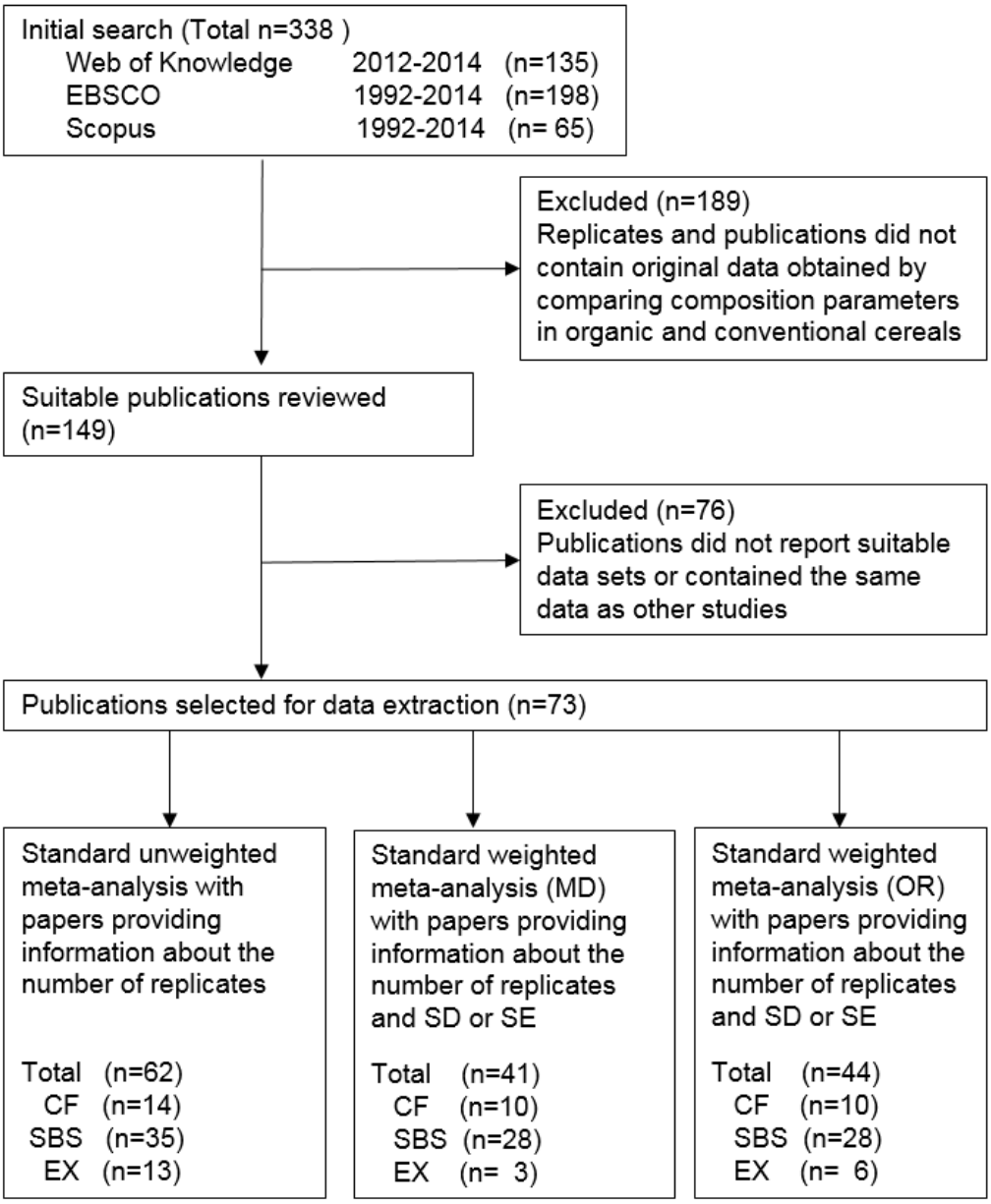


Figure 2.1 Summary of the search and selection protocols used to identify papers included in the meta-analysis. SD, standard deviation; SE, standard error; CF, comparison of matched farms; SBS, shopping basket studies; EX, controlled field experiments.

Approximately 90% of all studies included in the meta-analysis were carried out in Europe, mostly in Germany, Poland, Italy, Spain, Switzerland, Turkey, and Denmark (Appendix 2.3). Publications reported data on 28 different mycotoxins, 8 of which were included in the meta-analysis (Tables 2.5, 2.6 and 2.7). For the other 20 mycotoxins less than three comparative data-points were available and were therefore not included in the meta-analyses as recommended by Wang *et al.* (2018).

A total of seven different meta-analyses were undertaken, including two standard weighted analyses with mean difference (MD) and odds ratio (OR) as effect sizes, one standard unweighted analysis and four sensitivity analyses (Table 2.4). Sensitivity analyses included (a) a standard weighted meta-analysis without inclusion of data from poor and acceptable publications and (b) a standard weighted meta-analysis using averaged mycotoxin concentration and proportion by years instead of ones not averaged (data of single experimental year) when studies were carried more than 2 years and both averaged and not averaged data was reported.

For all mycotoxins with data-sets available (at least three comparative data-points) to analysise, results of (a) unweighted and weighted (MD and OR) meta-analysis (Tables 2.5, 2.6 and 2.7); (b) concentration and proportion changes over time (Figure 2.2 for DON and OTA, Appendix 2.4 for ZEA, total T-2/HT-2, total aflatoxins and total fumonisins); (c) GRADE assessment of strength of evidences for standard weighted meta-analysis with MD as the effect size (Table 2.12), were presented. For mycotoxins for which larger data-sets were available (DON, ZEA, and OTA) and where the standard meta-analyses detected significantly different concentrations between organic and conventional samples, (a) forest plots (Figures 2.3 and 2.4 for DON, Figures 2.5 and 2.6 for OTA, and figure 2.7 for ZEA) to further describe the variation between studies; and results of (b) multi-level analysis (Tables 2.8 and 2.10) for DON and OTA, respectively) and (c) the sensitivity analyses (Tables 2.9 and 2.11 for DON and OTA, respectively) were prepared.

2.4.1 Fusarium mycotoxins

Substantial amounts of data (n>50) were only available for one of the mycotoxins (deoxynivalenol, DON) produced by *Fusarium* species in cereal grains. DON is also the trichothene mycotoxin that is most commonly tested for in commercial quality assurance practice.

Both unweighted and weighted meta-analysis comparing DON concentrations (Tables 2.5 and 2.6) and the weighted odds-ratio (OR) meta-analyses of samples testing positive for DON (Table 2.7) detected significantly (a) higher DON concentrations and (b) proportions of samples testing positive for DON in conventional compared with organic cereal grains and

products. Mean DON concentrations were estimated to be ~80% or ~40% higher in conventional than organic cereals/cereal products in the weighted and unweighted meta-analyses respectively, when data from all years were considered (Tables 2.5 and 2.6). Also, approximately 25% more conventional than organic cereal grain/product samples tested positive for DON in the weighted OR meta-analysis (Table 2.7). However, it should be pointed out that heterogeneity was found to be very high, with I^2=96.57% for MD and I^2=50.81% for OR (Table 2.6 and 2.7).

When separate meta-analysis were carried out for different time periods (= data from studies carried out before 2004, between 2004 and 2009, and between 2010 and 2015) results indicated that the mean difference in (a) DON concentrations and (b) proportions of samples testing positive for DON between organic and conventional cereal grains/products decreased over time (Tables 2.5 to 2.7). Also, meta-analyses detected no significant differences between production systems for the last 6 year period (2010 to 2015) assessed. Regression analyses for both (a) DON concentrations and the (b) proportion of samples testing positive for DON showed the same trend (Figure 2.2). However, it should be pointed out that for the 2010 to 2015 period the evidence base was very small (only 3 studies and 9 comparative data sets) (Tables 2.5 to 2.7).

Weighted, multilevel model meta-analyses were carried out for to identify potential confounding effects of cereal species, climatic zone, country, study-type and mycotoxin analysis method on the difference in DON concentrations between organic and conventional cereal grains/products. Using the multilevel model significant differences between production systems could only be detected for specific (a) cereal species (wheat and rye), (b) climatic zones (Dfb and CfB), (c) countries (Germany, Austria, Belgium and Poland), (d) study types (Retail/basket and farm surveys) and analyses methods (HPLC) and in all cases significantly higher concentrations and/or proportions of positive samples were found in conventional cereal grains/products (Table 2.8). However, it should be pointed out that significant differences were primarily detected for cereal species, climatic zones and countries for which a larger number of studies/comparative DON data were available (Table 2.8).

The number of studies reporting data for all other *Fusarium* mycotoxins was relatively small (3-10) (Tables 2.5, 2.6 and 2.7), but the unweighted MD and weighted OR meta-analyses detected similar trends to those identified for DON for most other *Fusarium* mycotoxins. The unweighted meta-analysis detected significantly higher HT-2 Fusariotoxin (HT-2), total T-2/HT-2 Fusariotoxin and zeraleone (ZEA) concentrations, and a trend (P=0.096) towards higher concentrations of T-2 Fusariotoxin (T-2) in conventional compared with organic cereal grains/products (Table 2.5). The OR weighted meta-analysis identified significantly higher numbers of samples testing positive for HT-2, H-2 and ZEA in conventional compared with

organic cereal grain/products (Tables 2.7). When the OR for ZEA in different cereal species were compared numerically lower numbers of positive samples were detected in organic cereal grains/products of all 4 species assessed (wheat, barley, oats, rye), but the difference between production systems was only significant for rye (Table 2.7 and Figure2.7). In contrast, the weighted MD meta-analysis did not detect significant differences between production systems for *Fusarium* mycotoxins other than DON (Table 2.6). Also regression analyses indicate that concentrations of zeraleone, fumonisins, and total T-2/HT-2 mycotoxins decreased over time and have become very similar in the most recent time periods assessed (Appendix 2.4).

2.4.2 Common mould mycotoxins

Substantial amounts of comparative data (n>50) were only available for ochratoxin A, (OTA) which is produced by common mould fungi (*Penicillium* and *Aspergillus* spp.).

Both unweighted and weighted meta-analysis comparing OTA concentrations (Tables 2.5 and 2.6) and the weighted odds-ratio (OR) meta-analyses (Table 2.7)of samples testing positive for OTA detected significantly (a) higher OTA concentrations and (b) proportions of samples testing positive for OTA in organic compared with conventional cereal grains and products. Based on the unweighted meta-analysis, mean OTA concentrations were 92% higher in organic compared with conventional cereal grains/products when data from all years were considered (Table 2.5).

Similarly, approximately 25% more organic than conventional cereal grain/product samples tested positive for OTA in the weighted OR meta-analysis (Table 2.7). However, it should be pointed out that heterogeneity was found to be very high, with I^2=100% for MD and I^2=36% for OR (Table 2.6 and 2.7).

When separate meta-analysis were carried out for different time periods (= data from studies carried out before 2004, between 2004 and 2009, and between 2010 and 2015) results indicated that the mean difference in (a) OTA concentrations and (b) proportions of samples testing positive for OTA between organic and conventional cereal grains/products decreased over time (Tables 2.6 and 2.7). Also, the unweighted MD and weighted OR meta-analyses detected no significant difference between production systems for the last 6 year period (2010 to 2015) (Tables 2.5 and 2.7). Regression analyses indicate that OTA contamination in organic samples decreased over time, while it remained at similar levels in conventional samples, thus resulting in similar contamination levels in the most recent time period (2010 to 2015) assessed (Figure 2.2). However, it should be pointed out that for the 2010 to 2015 period the evidence base was very small (only 3 studies and 7 comparative data sets) (Tables 2.5 to 2.7).

Using the multilevel model significant differences between production systems could only be detected for specific (a) cereal species (rye, rice and barley), (b) climatic zones (Dfb and Csa), (c) countries (Denmark and Poland) and analyses methods (HPLC) and in all cases significantly higher concentrations and/or proportions of positive samples were found in organic cereal grains/products (Table 2.10). However, it should be pointed out that significant differences were primarily detected for cereal species, climatic zones and countries for which a large number of studies/comparative DON data was available (Table 2.10). It is also important to note that no significant difference in OTA contamination between organic and conventional cereal grains/products could be detected for common wheat (*Triticum sativum*) although a relatively large number of comparative data (n=30) was available (Table 2.10).

The number of studies reporting data for aflatoxins (AF; another mycotoxins produced by mould fungi) was relatively small (<10). Aflatoxins could only be detected in 1% of organic and 7% of conventional samples and estimated total AF concentrations very low and similar in organic and conventional cereal grains/products (Tables 2.5, 2.6 and 2.7). Correlation analyses suggest that aflatoxin concentrations decreased over time in both organic and conventional cereal grains/products (Appendix 2.4).

2.4.3 Ergot mycotoxins (Claviceps purpurea and other Claviceps spp.)

Since only two publications (Lauber *et al.*, 2005; Malysheva *et al.*, 2014) reported comparative data on ergot mycotoxin concentrations in conventional and organic cereal grains/products, meta-analyses were not carried out. However, these studies showed the frequencies and level of ergot alkaloids contamination in cereals were significantly lower in organic compared with conventional samples (Lauber *et al.*, 2005; Malysheva *et al.*, 2014).

2.4.4 Strength of Evidence

The overall assessment of the strength of evidence using an adapted GRADE (Grading of Recommendations, Assessments, Development and Evaluation) approach (Guyatt *et al.*, 2008) identified uncertainties in the evidence base, but there was little evidence for publication bias and the overall strength of evidence was high or moderate for the majority of parameters for which significant differences were detected (Table 2.12).

Table 2.4 Summary of inclusion criteria used in the standard unweighted (analysis 1) and the standard weighted (analysis 2 and 5) meta-analysis, and the 4 sensitivity analyses (3, 4, 6 and 7) carried out.

Analysis No	Result presenting form			Experimental years		Paper Quality	
	Only papers with N, mean, SE/SE	All papers reporting means	Paper reporting percentage of contaminated samples	One data point from one paper*	Individual year as separate data point**	All quality papers	Not including low and acceptable quality papers
Unweighted meta-analysis							
1 standard		+		+		+	
MD weighted meta-analysis							
2 standard	+			+		+	
3	+			+			+
4	+				+	+	
OR weighted meta-analysis							
5 standard			+	+		+	
6			+	+			+
7			+		+	+	

*If data from more than one experimental years were presented separately by year in the paper, average was calculated and included in the analysis;
**If data from more than one experimental years were presented separately by year in the paper, they were analysed separately, as individual data points;

Table 2.5 Results of the unweighted meta-analysis of mycotoxin contamination in organic vs conventional cereals and cereal based foods (a concentration of half the limit of detection was used when concentrations were below the limit of detection); MD, difference in mycotoxin concentration between organic and conventional samples.

				Organic (µg kg^{-1})			Conventional (µg kg^{-1})				
Parameter	**N**	**n**	***P***	**Mean**	**95% CI**		**Mean**	**95% CI**		**MD**	**Ratio**
***Fusarium* mycotoxins**											
Trichothenes											
DON (before 2004)	16	36	**<0.001**	94	61	127	183	25	163	-89	59
DON (2004-2009)	12	28	**<0.001**	122	44	199	196	16	228	-74	45
DON (2010-2015)	3	9	0.412	99	-0.4	199	161	-104	302	-62	96
DON (all years)	29	73	**<0.001**	105	70	141	185	48	163	-80	57
NIV	7	10	0.126	23	4	41	37	-7	52	-14	69
HT-2	7	11	**0.035**	19	5	33	24	4	34	-6	58
T-2	6	9	0.096	18	2	33	22	0	35	-4	67
Total T-2/HT-2*	10	24	**<0.001**	26	6	46	50	-29	81	-23	57
Zearalones											
ZEA	14	37	**0.010**	5	3	7	9	0	9	-4	67
Fumonisins											
Total Fumonisins**	3	18	0.312	69	44	95	61	49	89	8	107
Mould mycotoxins											
(Penicillium/Aspergillus)											
Ochratoxin A											
OTA (before 2004)	8	41	0.066	3.1	1.1	5.1	1.4	1.8	4.3	1.7	149
OTA (2004-2009)	7	29	**0.010**	1.9	0.2	3.6	1.4	0.2	3.6	0.5	259
OTA (2010-2015)	3	7	0.135	1.1	0.8	1.4	1.0	0.6	1.6	0.1	159
OTA (all years)	17	77	**0.002**	2.5	1.2	3.7	1.3	1.6	3.4	1.1	184
Aflatoxins											
Total aflatoxins***	9	22	0.303	0.86	-0.04	1.77	0.86	-0.04	1.77	0.00	117

N, number of publication included in comparison; n, number of data points included in the comparison; MD, mean difference between mycotoxin concentrations in organic and conventional samples; 95% CI, 95% confidence intervals; DON, deoxynivalenol; NIV, nivalenol; HT-2, HT-2 Fusariotoxin; T-2, T-2 Fusariotoxin; ZEA, zearalenone; OTA, ochratoxin A; * Total T-2/HT-2 includes HT-2, T-2, T-2 tetraol and T-2 triol; ** Total Fumonisins include data for fumonisin B_1 and fumonisin B_2 determined by HPLC/GC and fumonisins determined by an ELISA method; *** Total aflatoxins include aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2).

Table 2.6 Results of the weighted meta-analysis of mycotoxin contamination in organic vs conventional cereals and cereal based foods (a concentration of half the limit of detection was used when concentrations were below the limit of detection); MD, difference in mycotoxin concentration (µg kg^{-1}) between organic and conventional samples.

Parameter	N	n	*P*	MD	Heterogeneity (I^2-value)	95% CI	
***Fusarium* mycotoxins**							
Trichothenes							
DON (before 2014)	16	36	**<0.001**	-48	74	-75	-21
DON (2004-2009)	12	28	**0.009**	-40	99	-71	-10
DON (20010-2015)	3	9	0.419	3	0	-5	12
DON (all years)	29	73	**<0.001**	-40	96	-58	-22
NIV	7	10	0.459	1.5	83	-2.4	5.3
HT-2	7	11	0.144	-4.7	70	-11.1	1.6
T-2	6	9	0.973	0.0	0	-0.2	0.1
Total T-2/HT-2*					Not available		
Zearalones							
ZEA	14	37	0.155	-0.2	29	-0.6	0.1
Fumonisins							
Total fumonisins**	3	18	0.392	5	3	-6	15
Mould mycotoxins							
(Penicillium/Aspergillus)							
Ochratoxin A (OTA)							
OTA (before 2004)	8	41	0.119	0.1	93	-0.03	0.22
OTA (2004-2009)	7	23	**0.036**	0.7	100	0.05	1.28
OTA (2010-2015)	3	7	**0.001**	0.4	0	0.18	0.70
OTA (all years)	17	71	**0.002**	0.5	100	0.19	0.80
Aflatoxins							
Total aflatoxins***	9	22	0.647	0.01	91	-0.02	0.04

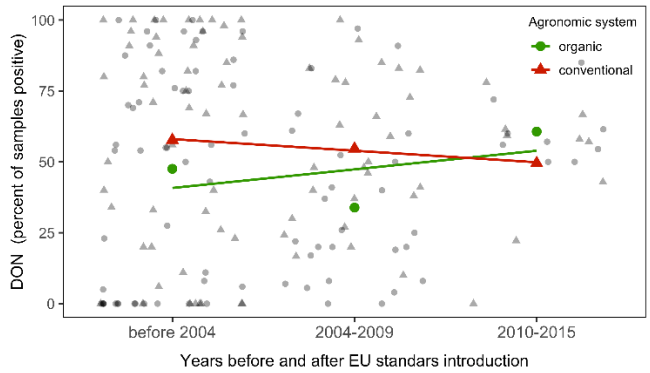
N, number of publication included in comparison; n, number of data points included in the comparison; 95% CI, 95% confidence intervals; DON, deoxynivalenol; NIV, nivalenol; HT-2, HT-2 Fusariotoxin; T-2, T-2 Fusariotoxin; ZEA, zearalenone; OTA, ochratoxin A; * Total T-2/HT-2 includes HT-2, T-2, T-2 tetraol and T-2 triol; ** Total Fumonisins include data for fumonisin B_1 and fumonisin B_2 determined by HPLC/GC and fumonisins determined by an ELISA method; *** Total aflatoxins include aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2).

Table 2.7 Results of the standard weighted odds ratios (OR) of mycotoxin contamination (proportion of positive samples) in organic vs conventional cereals and cereal based foods. OR values <0 indicate higher concentration and proportions of positive samples in conventional cereals/cereal products; OR values>0 indicate higher concentration and proportions of positive samples in organic cereals/cereal products

Parameter	N	n	OR	% positive samples		*P*-value	Heterogeneity (I^2-value)	95% CI	
				organic	conventional				
***Fusarium* mycotoxins**									
Trichothenes									
DON (before 2004)	17	48	-0.74	48	58	**<0.001**	22	-1.01	-0.48
DON (2004-2009)	11	26	-1.03	34	55	**<0.001**	73	-1.62	-0.43
DON (2010-2015)	3	9	0.30	61	50	0.285	0	-0.25	0.84
DON (all years)	29	83	-0.73	45	56	**<0.001**	51	-1.00	-0.47
NIV	9	16	0.21	23	15	0.593	42	-0.57	0.99
HT-2	10	26	-0.69	14	18	**0.010**	3	-1.22	-0.16
T-2	9	26	-0.71	13	18	**0.011**	11	-1.26	-0.16
Total T-2/HT-2*	not available								
Fumonisins									
FUM	1	6	0.13	34	35	0.718	0	-0.57	0.83
Total fumonisins**	not available								
Zearalones									
ZEA	12	34	-0.43	20	27	**0.033**	29	-0.82	-0.03
Mould mycotoxins *(Penicillium/Aspergillus)*									
Ochratoxin A									
OTA (before 2004)	8	43	0.52	60	51	**0.019**	51	0.09	0.95
OTA (2004-2009)	6	27	NA	25	11	NA	0	NA	NA
OTA (2010-2015)	3	9	0.39	32	28	0.281	0	-0.32	1.09
OTA (all years)	16	79	0.52	44	35	**0.001**	36	0.21	0.84
Aflatoxins (AF)									
AF-B1	6	11	-0.38	1	7	0.492	0	-1.47	0.71
Total aflatoxins***	not available								

N, number of publication included in comparison; n, number of data points included in the comparison; MD, mean difference; 95% CI, 95% confidence intervals; DON, deoxynivalenol; NIV, nivalenol; HT-2, HT-2 Fusariotoxin; T-2, T-2 Fusariotoxin; FUM, fumonisins (determined by a HPLC methods); ZEA, zearalenone;; OTA, ochratoxin A; AF, aflatoxin ** Total Fumonisins include data for fumonisin B_1 and fumonisin B_2 determined by HPLC/GC and fumonisins determined by an ELISA method; *** Total aflatoxins include aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1) and aflatoxin G_2 (AFG_2).

2a



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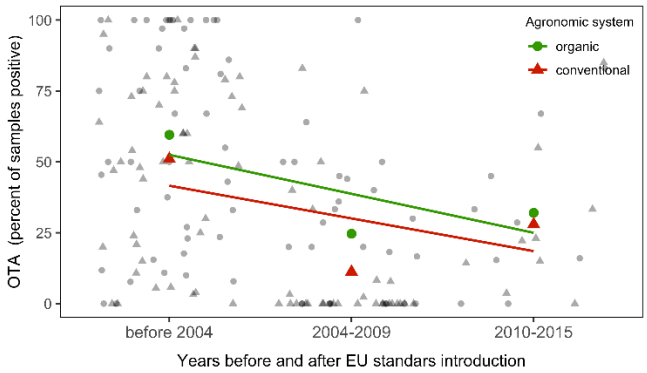

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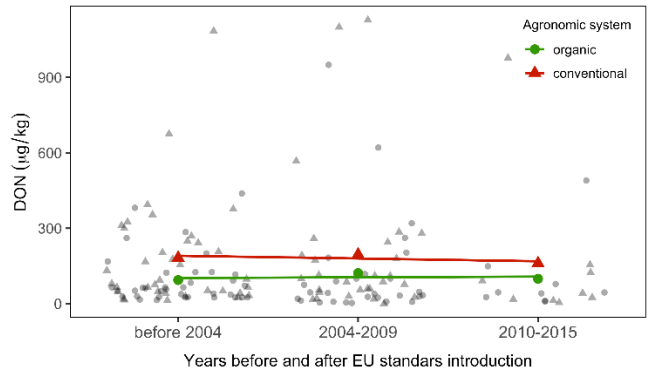

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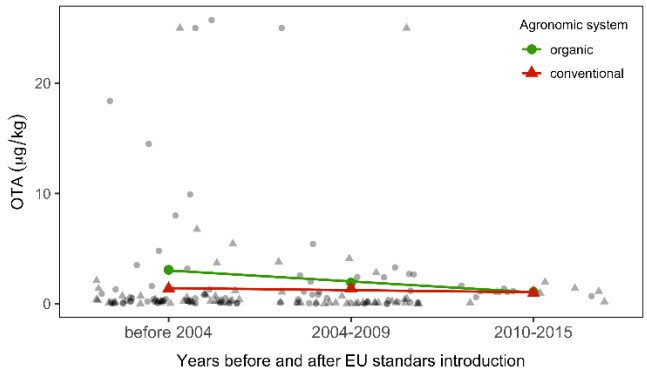


Figure 2.2 Proportions of positive samples and mean concentration of DON and OTA found in organic and conventional cereals and cereal products in comparative studies carried out between 1992-2015. First order regressions lines are between means of data from studies carried out before 2004, between 2004 and 2009 and between 2010 and 2015 and indicate changes in organic (green lines) and conventional (red lines). Grey circle and triangles represent individual data points for organic and conventional samples respectively from all studies included in the unweighted meta-analyses. 2a, proportion of samples testing positive for DON; 2b. Proportion of samples testing positive for OTA; 2c, mean DON concentration; 2d, mean OTA concentration.

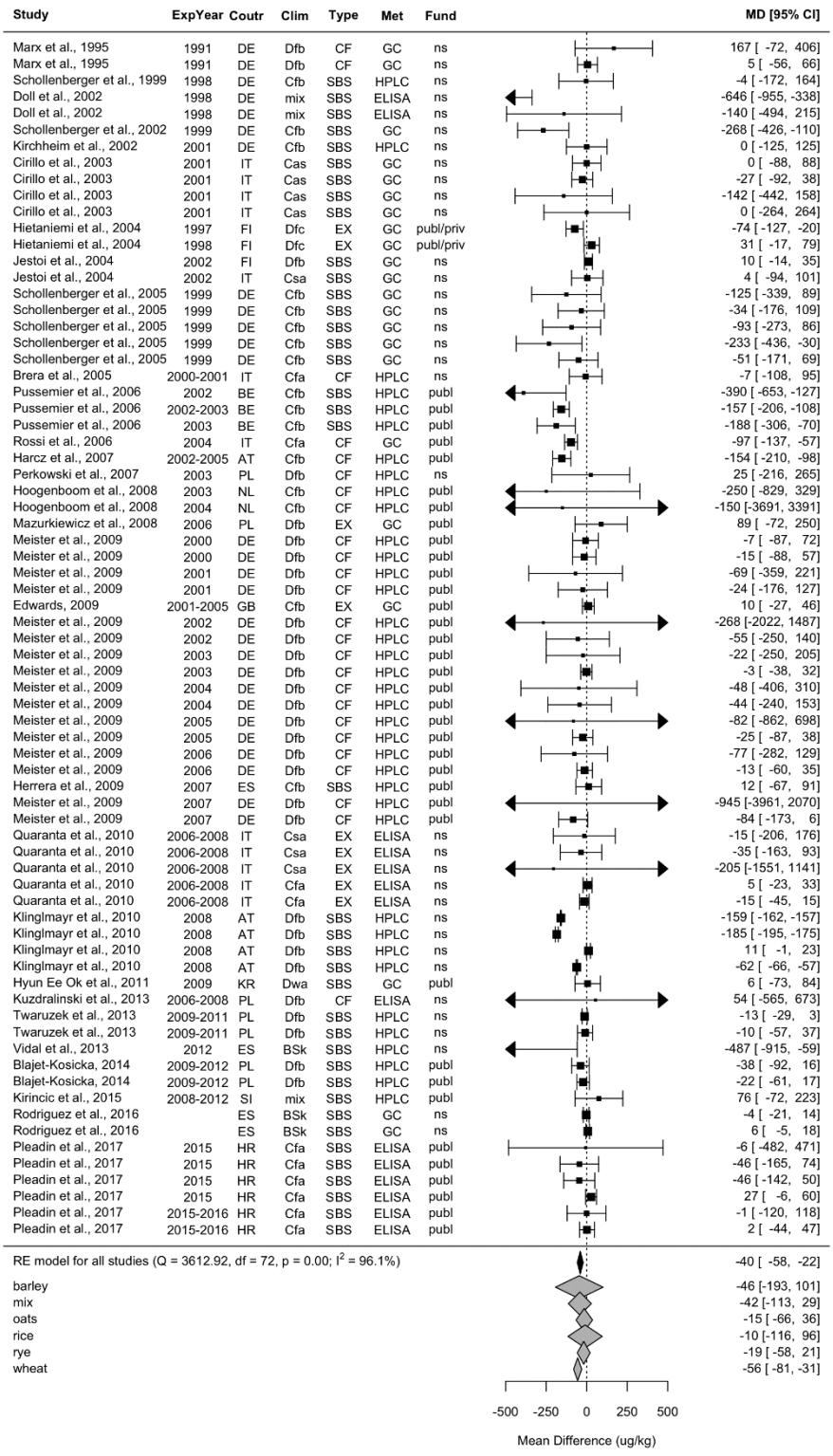


Figure 2.3 Forest plot showing the results of the comparison of **DON** content between organic and conventional cereals and cereals products using **mean differences (MD)** with 95% confidence intervals (95% CI), for studies included in the standard weighted meta-analysis. The estimated average MD from random-effect (RE) model for all studies and MDs for different cereal species are indicated at the bottom of the figure from control group (conventional). Sign of the MD indicates if the analysed parameter is higher (+) or lower (-) in organic cereals. SBS-shopping basket study; CF-comparison of farms; EX-controlled experiment.

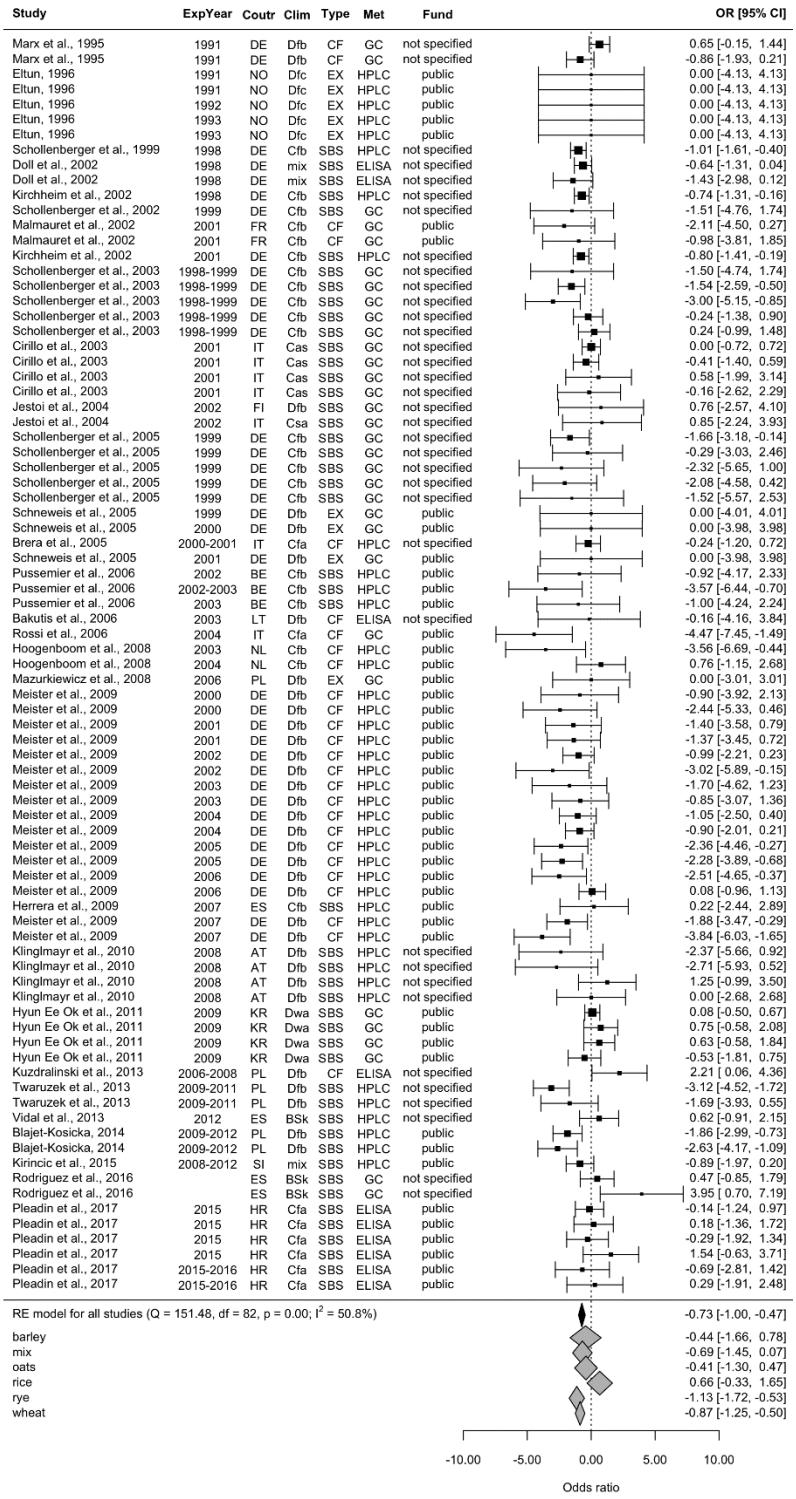


Figure 2.4 Forest plot showing the results of the comparison of **DON** occurrence frequency between organic and conventional cereals and cereals products using **odds ratio (OR)** with 95% confidence intervals (95% CI), for studies included in the standard weighted meta-analysis. The estimated average OR from random-effect (RE) model for all studies and ORs for different product groups are indicated at the bottom of the figure from control group (conventional). Sign of the OR indicates if the analysed parameter is higher (+) or lower (-) in organic cereals. SBS-shopping basket study; CF-comparison of farms; EX-controlled experiment.

Table 2.8 Effect of cereal species, climate, country, study type and analysis method on DON contamination in organic vs conventional cereals and cereal based foods; results of a multi-level, weighted meta-analyses determining mean differences (MD) for DON concentrations and odds ratios (OD) for the proportion of samples testing positive for DON contamination.

Analyses/Factors	**n**	**MD**	***P***	**95% CI**		**n**	**OR**	***P***	**95% CI**	
Random-Effect Model*	73	-40	**<0.001**	-58	-22	83	-0.73	**<0.001**	-1	-0.47
Multilevel Model	73	-40	**0.005**	-67	-12	83	-0.62	**0.001**	-0.98	-0.26
Species										
Wheat	42	-56	**<0.001**	-81	-31	44	-0.87	**<0.001**	-1.25	-0.5
Rye	14	-19	0.358	-58	21	14	-1.13	**<0.001**	-1.72	-0.53
Oats	7	-15	0.558	-66	36	8	-0.41	0.363	-1.3	0.47
Rice	2	-10	0.856	-116	96	5	0.66	0.19	-0.33	1.65
Barley	1	-46	0.539	-193	101	5	-0.44	0.478	-1.66	0.78
Mix	7	-42	0.244	-113	29	7	-0.69	0.076	-1.45	0.07
Climate										
Dfb	30	-35	**0.016**	-63	-6	33	-1.16	**<0.001**	-1.56	-0.76
Cfb	16	-97	**<0.001**	-141	-53	22	-1.04	**<0.001**	-1.51	-0.58
BSk	3	-14	0.725	-94	65	3	0.97	0.122	-0.26	2.19
Cas	4	-15	0.775	-115	85	4	-0.1	0.831	-1.02	0.82
Cfa	10	-18	0.413	-62	26	8	-0.23	0.541	-0.95	0.5
Csa	4	-23	0.609	-111	65	1	0.85	0.616	-2.46	4.16
Dfc	2	-21	0.646	-108	67	5	0	1	-1.92	1.92
Dwa	1	6	0.935	-132	143	4	0.21	0.609	-0.59	1.01
Mix	3	-105	0.163	-254	43	3	-0.89	0.057	-1.82	0.03
Country										
Germany	28	-42	**0.009**	-73	-11	37	-1.03	**<0.001**	-1.34	-0.71
Austria	5	-107	**<0.001**	-147	-67	4	-0.51	0.504	-1.99	0.98
Belgium	3	-185	**<0.001**	-264	-106	3	-1.96	**0.042**	-3.86	-0.07
Spain	4	-4	0.89	-58	51	4	0.84	0.13	-0.24	1.92
Finaland	3	-8	0.769	-64	47	1	0.76	0.669	-2.73	4.26
Croatia	6	-4	0.868	-56	47	6	0.07	0.865	-0.74	0.88
Italy	12	-25	0.191	-63	13	7	-0.3	0.389	-1	0.39
South Korea	1	6	0.924	-111	122	4	0.21	0.588	-0.54	0.95
Netherlands	2	-247	0.401	-825	330	2	-0.55	0.55	-2.37	1.26
Poland	7	-12	0.617	-57	34	6	-1.67	**<0.001**	-2.49	-0.84
Slovenia	1	76	0.386	-95	246	1	-0.89	0.248	-2.39	0.62
UK	1	10	0.842	-84	103					
Norway						5	0	1	-1.91	1.91
France						2	-1.63	0.105	-3.6	0.34
Lithuania						1	-0.16	0.939	-4.29	3.97
Study Type										
Shopping Basket	39	-50	**<0.001**	-73.71	-26.28	48	-0.6	**<0.001**	-0.93	-0.27
Matched Farms	25	-37	**<0.044**	-73.62	-1.04	26	-1.09	**<0.001**	-1.57	-0.62
Experiment	9	-5	0.843	-52.48	42.85	9	0	1	-1.4	1.4
Method										
ELISA	14	-21	0.338	-64.33	22.09	10	-0.1	0.759	-0.75	0.55
GC	22	-24	0.128	-53.93	6.81	32	-0.35	0.071	-0.74	0.03
HPLC	37	-57	**<0.001**	-81.78	-31.91	41	-1.18	**<0.001**	-1.52	-0.83

* random effects model was used for the meta-analyses presented in tables 2.6 and 2.7; n, number of data points included in the comparison; MD, mean difference in mycotoxin concentrations between organic and conventional samples; 95% CI, 95% confidence intervals; OR, odd ration for positive samples; MD and OR values <0 indicate higher concentration and proportions of positive samples in conventional cereals/cereal products; MD and OR values >0 indicate higher mycotoxin concentration and proportions of positive samples in organic cereals/cereal products

Table 2.9 Effect of removing poor quality data or data from more than two years on the results of standard weighted mean difference and odds-ratio meta-analyses performed; outcomes of the sensitivity analyses carried out for DON.

Analyses/Factors	**n**	**MD**	***P***	**95% CI**		**n**	**OR**	***P***	**95% CI**	
Standard analyses										
Random-Effect Model	73	-40	**<0.001**	-58	-22	83	-0.73	**<0.001**	-1	-0.47
Multilevel Model	73	-40	**0.005**	-67	-12	83	-0.62	**0.001**	-0.98	-0.26
Sensitive Analysis										
Effect of removing poor and acceptable quality publications										
Std Random-Effect Model	70	-42	**<0.001**	-60.61	-23.8	66	-0.75	**<0.001**	-1.05	-0.45
Multilevel Model	70	-43	**0.0038**	-75.04	-14.37	66	-0.64	**0.005**	-1.08	-0.19
Effect of removing average data from more than two years			Removing average data from more than two years							
Std Random-Effect Model	56	-40	**<0.001**	-60.34	-19.17	59	-0.55	**0.001**	-0.85	-0.25
Multilevel Model	56	-40	**0.007**	-63.33	-10.23	59	-0.61	**0.004**	-1.03	-0.2

n, number of data points included in the comparison; MD, mean difference in mycotoxin concentrations between organic and conventional samples; 95% CI, 95% confidence intervals; OR, odd ration for positive samples; MD and OR values <0 indicate higher concentration and proportions of positive samples in conventional cereals/cereal products; MD and OR values >0 indicate higher mycotoxin concentration and proportions of positive samples in organic cereals/cereal products

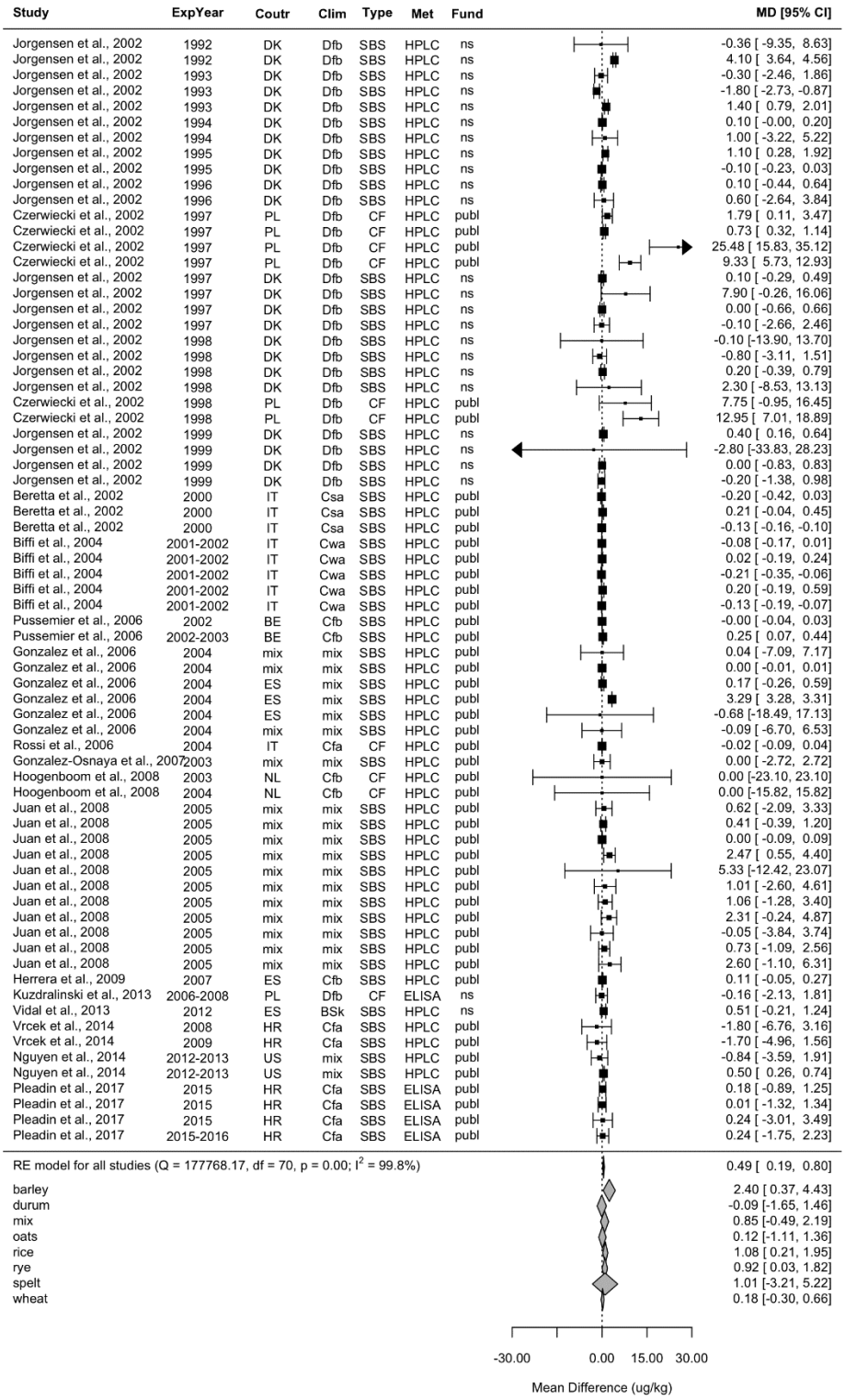


Figure 2.5 Forest plot showing the results of the comparison of **OTA** content between organic and conventional cereals and cereals products using **mean differences (MD)** with 95% confidence intervals (95% CI), for studies in the standard weighted meta-analysis. The estimated average MD from random-effect (RE) model for all studies and MDs for different product groups are indicated at the bottom of the figure from control group (conventional). Sign of the MD indicates if the analysed parameter is higher (+) or lower (-) in organic cereals. SBS-shopping basket study; CF-comparison of farms; EX-controlled experiment.

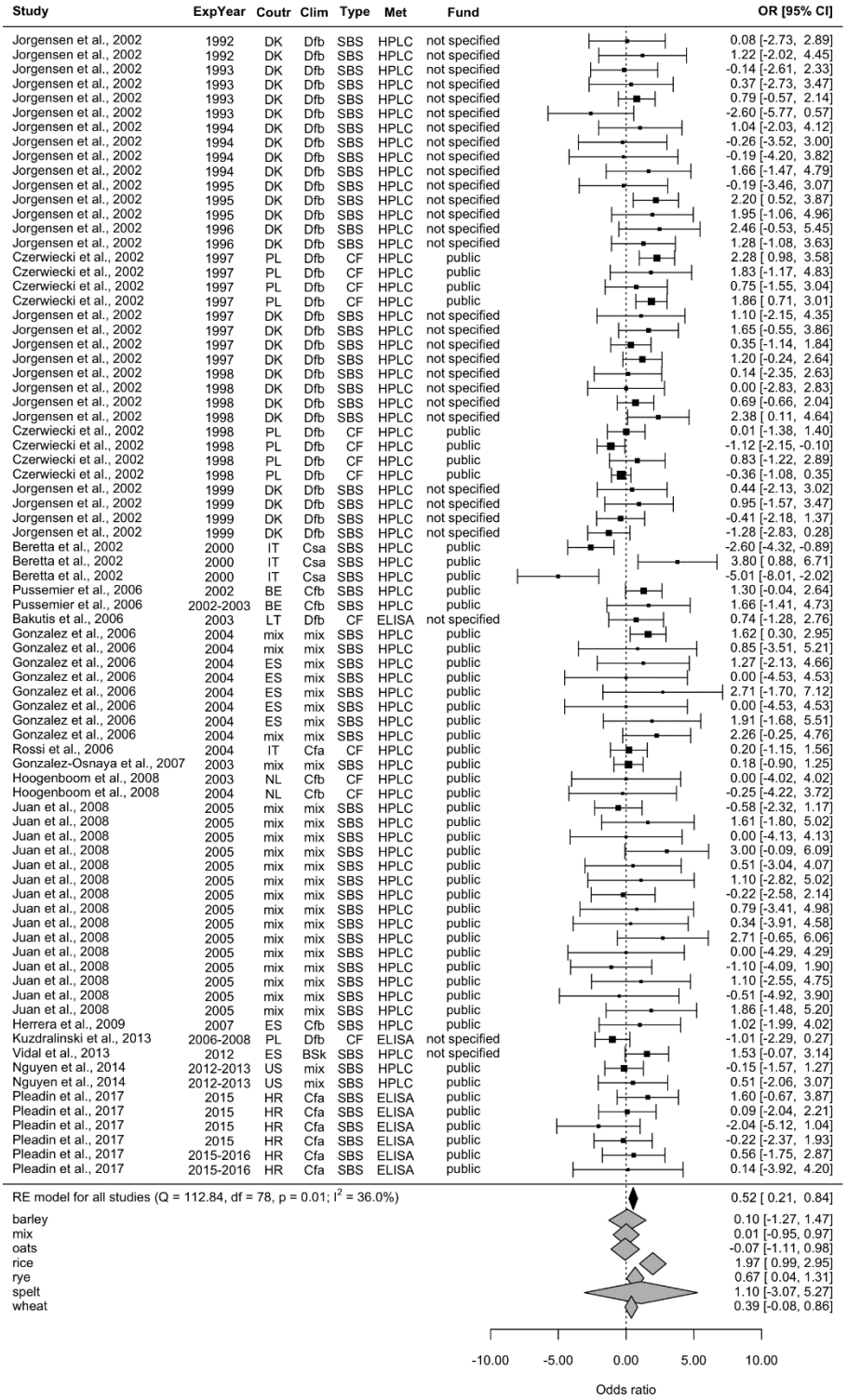


Figure 2.6 Forest plot showing the results of the comparison of ochratoxin (**OTA**) occurrence frequency between organic and conventional cereals and cereals products using **odds ratios (OR)** with 95% confidence intervals (95% CI), for studies in the standard weighted meta-analysis. The estimated average OR from random-effect (RE) model for all studies and ORs for different product groups are indicated at the bottom of the figure from control group (conventional). Sign of the OR indicates if the analysed parameter is higher (+) or lower (-) in organic cereals. SBS-shopping basket study; CF-comparison of farms; EX-controlled experiment.

Table 2.10 Effect of cereal species, climate, country, study type and analysis method on OTA contamination in organic vs conventional cereals and cereal based foods; results of a multi-level, weighted meta-analyses determining mean differences (MD) for OTA concentations and odds ratios (OD) for the proportion of samples testing positive for OTA contamination.

Analyses/Factors	**N**	**MD**	***P*-value**	**95% CI**		**n**	**OR**	***P*-value**	**95% CI**	
Random-Effect Model*	71	0.49	0.002	0.19	0.80	79	0.52	0.001	0.21	0.84
Multilevel Model	71	0.30	0.046	0.01	0.60	79	0.41	0.106	-0.09	0.91
Species										
Wheat	30	0.18	0.458	-0.30	0.66	30	0.39	0.107	-0.08	0.86
Rice	11	1.08	0.015	0.21	1.95	12	1.97	<0.001	0.99	2.95
Rye	15	0.92	0.043	0.03	1.82	19	0.67	0.038	0.04	1.31
Barley	3	2.40	0.020	0.37	4.43	6	0.10	0.890	-1.27	1.47
Spelt	5	0.12	0.846	-1.11	1.36	1	1.10	0.606	-3.07	5.27
Oats	1	1.01	0.640	-3.21	5.22	7	-0.07	0.902	-1.11	0.98
Durum	2	-0.09	0.907	-1.65	1.46					
Mix	4	0.85	0.214	-0.49	2.19	4	0.01	0.984	-0.95	0.97
Climate										
Dfb	30	0.76	0.003	0.26	1.26	37	0.54	0.009	0.14	0.95
BSk	1	0.51	0.640	-1.64	2.67	1	1.53	0.160	-0.61	3.67
Cfa	7	-0.11	0.833	-1.17	0.94	7	0.17	0.746	-0.84	1.18
Cfb	5	0.12	0.843	-1.05	1.29	5	1.02	0.137	-0.32	2.37
Csa	3	-0.04	0.947	-1.22	1.14	3	-1.60	0.050	-3.21	0.00
Cwa	5	-0.04	0.930	-0.95	0.87					
Mix	20	0.85	0.011	0.19	1.50	26	0.72	0.023	0.10	1.35
Country										
Denmark	23	0.40	0.161	-0.16	0.97	27	0.65	0.018	0.11	1.19
Belgium	2	0.12	0.872	-1.38	1.62	2	1.39	0.119	-0.36	3.14
Spain	5	1.04	0.059	-0.04	2.12	7	1.32	0.052	-0.01	2.65
Croatia	6	-0.16	0.810	-1.42	1.11	6	0.15	0.808	-1.05	1.35
Italy	9	-0.04	0.916	-0.75	0.67	4	-0.88	0.177	-2.16	0.40
Netherlands	2	0.00	1.000	-13.15	13.15	2	-0.13	0.934	-3.15	2.90
Poland	7	2.78	<0.001	1.46	4.09	9	0.37	0.303	-0.33	1.07
US	2	0.13	0.885	-1.68	1.95	2	0.07	0.939	-1.64	1.78
Lithuania						1	0.74	0.567	-1.80	3.28
Mix	15	0.73	0.086	-0.10	1.55	19	0.78	0.040	0.04	1.52
Study Type										
Shopping Basket	61	0.37	0.032	0.03	0.70	66	0.58	0.002	0.22	0.95
Matched Farms	10	1.95	<0.001	0.85	3.04	13	0.36	0.269	-0.28	0.99
Analysis Method										
ELISA	5	0.09	0.881	-1.10	1.29	8	-0.03	0.946	-0.98	0.92
HPLC	66	0.52	0.001	0.20	0.84	71	0.59	<0.001	0.26	0.92

* random effects model was used for the meta-analyses presented in tables 2.6 and 2.7; n, number of data points included in the comparison; MD, mean difference in mycotoxin concentrations between organic and conventional samples; 95% CI, 95% confidence intervals; OR, odd ration for proportion of positive samples; MD and OR values <0 indicate higher mycotoxin concentration and proportions of positive samples in conventional cereals/cereal products; MD and OR values >0 indicate higher mycotoxin concentrations and proportions of positive samples in organic cereals/cereal products; Mix: more than one species/climate/ country were included in the study.

Table 2.11 Effect of removing poor and acceptable quality data or data from more than two years on the results of standard weighted mean difference and odds-ratio meta-analyses performed; outcomes of the sensitivity analyses carried out for OTA .

Analyses/ Factors	**n**	**MD**	***P*-value**	**95% CI**		**n**	**OR**	***P*-value**	**95% CI**	
Standard analyses										
Random-Effect Model	71	0.49	0.002	0.19	0.8	79	0.52	0.001	0.21	0.84
Multilevel Model	71	0.3	0.046	0.01	0.6	79	0.41	0.106	-0.09	0.91
Sensitive analysis										
Effect of removing poor and acceptable quality publications										
Std Random-Effect Model	63	1.28	0.008	0.33	2.22	71	0.45	0.007	0.12	0.78
Multilevel Model	63	0.2	0.071	-0.02	0.42	71	0.32	0.251	-0.19	0.83
Effect of removing average data from more than two years										
Random-Effect Model						50	0.49	0.02	0.06	0.91
Multilevel Model						50	0.4	0.16	-0.16	0.97

n, number of data points included in the comparison; MD, mean difference in mycotoxin concentrations between organic and conventional samples; 95% CI, 95% confidence intervals; OR, odd ration for proportion of positive samples; MD and OR values <0 indicate higher mycotoxin concentration and proportions of positive samples in conventional cereals/cereal products; MD and OR values >0 indicate higher mycotoxin concentrations and proportions of positive samples in organic cereals/cereal products

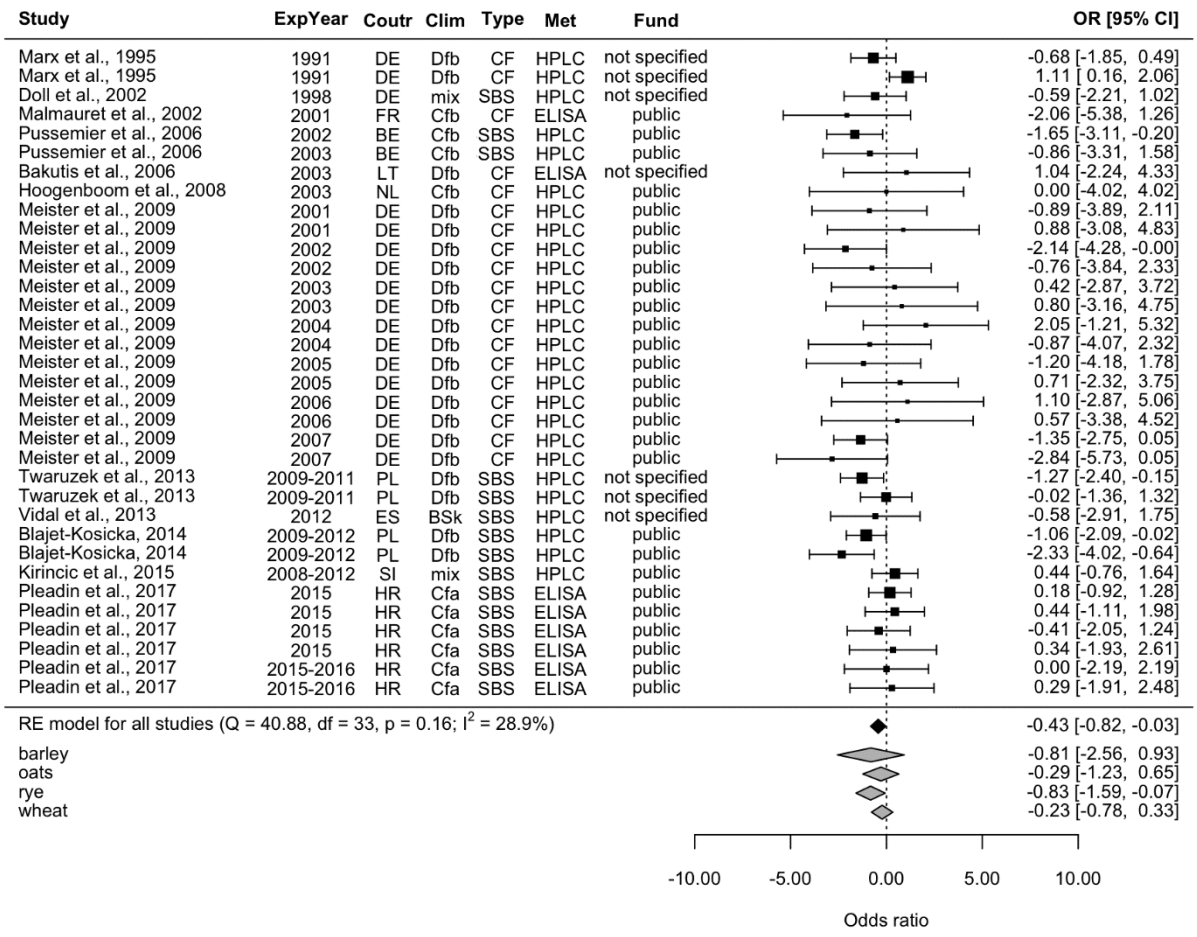


Figure 2.7 Forest plot showing the results of the comparison of zearalenone (**ZEA**) occurrence frequency between organic and conventional cereals and cereals products using mean differences (OR) with 95% confidence intervals (95% CI), for studies in the standard weighted meta-analysis. The estimated average OR from random-effect (RE) model for all studies and ORs for different product groups are indicated at the bottom of the figure from control group (conventional). Sign of the OR indicates if the analysed parameter is higher (+) or lower (-) in organic cereals. SBS-shopping basket study; CF-comparison of farms; EX-controlled experiment.

Table 2.12 GRADE (Grading of Recommendations, Assessments, Development and Evaluation) assessment of the strength of evidence for standard weighted meta-analysis with MD as effect size for all parameters included.

Parameter	Effect Magnitude	Precisions	Incon-sistency	Publication Bias	Overall Reliability
***Fusarium* mycotoxins**					
Trichothenes					
DON	Small	High	High	No	Moderate
3-AcDON	Moderate	High	Medium	No	Moderate
NIV	Small	High	Low	No	High
HT-2	Moderate	High	Medium	Medium	Moderate
T-2	Small	High	Medium	No	Moderate
Zearalones					
ZEA	Small	High	Medium	Medium	Moderate
Fumonisins					
FB1	Small	Low	Medium	No	Low
FB2	Small	Low	Medium	No	Low
FUM	Moderate	Medium	Medium	No	Moderate
Total fumonisins**	Moderate	Medium	Medium	No	Moderate
Mould mycotoxins *(Penicillium/Aspergillus)*					
Ochratoxin A (OTA)	Small	High	High	No	Moderate
AFB1	Small	High	High	No	Moderate
Total Aflatoxins***	Moderate	High	High	No	Moderate

DON, deoxynivalenol; 3-AcDON, 3-acetyldeoxynivalenol; NIV, nivalenol; HT-2, HT-2 Fusariotoxin; T-2, T-2 Fusariotoxin; ZEA, zearalenone; FB1, fumonisin B1 (determined by HPLC/GC); FB2, fumonisin B2 (determined by HPLC/GC); FUM, fumonisins (determined by a HPLC methods); ** Total Fumonisins include data for fumonisin B1 and fumonisin B2 determined by HPLC/GC and fumonisins determined by an ELISA method. OTA, ochratoxin A; AFB1, aflatoxin B1; *** Total aflatoxins include aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2).

Effect magnitude: Study quality was considered low because of high risks of bias and potential for confounding. However, we considered large effects to mitigate this GRADE; large effects were defined as 20%, moderate effects as 10-2-% and small as 10%.

Precision was based on the width of the polled effect CI and the extent of overlap in the substantive interpretation of effect magnitude GRADE.

Inconsistency was based on the measure of heterogeneity and the consistency of effect direction GRADE.

Publication bias was assessed using visual inspection of funel plots, Egger tests, two fail-safe number tests, and trim and fill. Overall publication bias was considered strong when indicated by two or more methods, moderated when indicated by one method, and low when indicated by none of the methods.

The overall quality of evident was then assessed across domains as in standard GRADE appraisal.

2.5 Discussion

In the early part of the 21th century a commentary in Nature (Trewavas, 2001b) suggested that organic farming practices result in higher levels of mycotoxin contamination in crops. The author concluded/claimed that "*Mycotoxins from contaminating fungi (which can be controlled by specific fungicides) definitely contribute to European cancer rates — fumonisin and patulin are both reported to be higher in organic products and failure to use effective fungicides on organic farms has led to these farms acting as repositories of disease"*.

The study was widely cited in the scientific literature and media and raised considerable concerns among consumers about the safety of organic food.

Consumers are increasingly aware about food safety issues and demand safe and nutritious food with minimal microbiological or chemical contaminants (Bhat *et al.*, 2010). This concern led to the use of some of the most toxic and environmentally damaging synthetic agrochemicals (ethylene dioxide, methyl bromide, organochlorines) to be banned in many developed and developing countries (Bhat *et al.*, 2010) and contributed to the increase in the demand for organic food products (Wier *et al.*, 2008; Yadav and Pathak, 2016).

Mycotoxin contamination of cereals and other crops was largely ignored as a global health issue (Wild and Gong, 2010), but is now recognised as an important food safety issue due to increased epidemiological evidence for harmful effects of mycotoxins on both human and animal health (Bryla *et al.*, 2016; Ferrigo *et al.*, 2016). Specifically, trichothecenes (such as DON and T-2 toxin) can suppress the immune system by inhibiting protein synthesis and cell proliferation. Ochratoxin A (OTA) can have chronic effects on health, including carcinogenicity, mutagenesis and immunosuppression (Bryla *et al.*, 2016; Ferrigo *et al.*, 2016).

Most studies comparing mycotoxin contamination in organic and conventional cereal grains/products used for the meta-analyses reported here focused on DON and OTA. However, sufficient data to carry out meta-analyses for a range of other *Fusarium* mycotoxins and aflatoxins were also available, but results need to be considered with caution, due to the relatively smaller evidence base for mycotoxins other than DON and OTA (Tables 2.5 to 2.7).

Results from this study showed that concentrations of all mycotoxins included in meta-analyses were lower than the maximum contamination levels (MCLs) set by the EU for cereals intended for direct consumption by adult humans in both organic and conventional cereal grains/products (Tables 2.5 to 2.7). OTA were closest to MCLs (1.0 and 1.1 for conventional and organic cereals respectively) which is (a) 3 times lower than the maximum OTA levels (3 µg/kg) set for older children and adults, but (b) twice the amount set as the maximum OTA level (0.5 µg/kg) for cereal based foods and baby foods for infants and young children (European Commission, 2006a).

Since *Fusarium* mycotoxin contamination is primarily affected by environmental conditions and agronomic practices per-harvest, while contamination with common mould mycotoxins is more closely associated with post-harvest grain treatment, storage and quality assurance practices, results obtained for *Fusarium* and mould mycotoxins are discussed in separate sections below .

2.5.1 Fusarium mycotoxins

Guidelines to minimise the risk of *Fusarium* mycotoxins in cereals include the use of fungicides for (a) seed treatment and (b) the control of stem-base fungal disease to prevent lodging (which increases the risk of *Fusarium* grain infection from soil surface inocula) and (c) foliar sprays to minimise infection of grains from *Fusarium* infected leaves (AHDB, 2006)

Fungicide seed and foliar treatments are prohibited under organic farming standards and the non-use of fungicides was claimed to result in a higher levels of mycotoxin contamination in organic cereals and other crops in some scientific reviews/commentaries over the last 20 years (Trewavas, 2001b). By showing that overall *Fusarium* mycotoxin contamination was lower in organic than conventional cereal/cereal products, the meta-analyses reported here contradict the conclusion of these reviews (Tables 2.5, 2.6 and 2.7). However, the results presented here are consistent with a range of qualitative reviews (Benbrook, 2005; Gottschalk *et al.*, 2007; Brodal *et al.*, 2016b) and the only previous meta-analysis of comparative DON contamination data (Smith-Spangler *et al.*, 2012), which reported significantly lower levels of DON in organic compared with conventional common wheat samples (SMD, -0.94 [CI, -1.27 to -0.62]; $P<0.01$; $I^2=63$).

These findings suggest that the preventative agronomic methods used by organic cereal producers are as, or possibly more, effective at reducing *Fusarium* mycotoxin contamination of cereal grains as the use fungicide treatment-based methods used in conventional production. This view is supported by studies which showed that a range of agronomic practices that are widely used in conventional production and increase mycotoxin risk are avoided by organic farmers (Dill-Macky and Jones, 2000; Krebs *et al.*, 2000; Eiblmeier and von Gleissenthall, 2007; Suproniene *et al.*, 2012). For example, a detailed review by Paulsen and Weißmann (2002a) identified 13 agronomic/farm management factors that may affect mycotoxin formation and contamination in food and feed crops from organic and/or conventional crop production systems. Three of the 13 factors were described to potentially increase the risk of mycotoxin contamination in organic systems. These included (1) the prohibition on fungicides application, (2) higher weed density (which may provide alternative hosts for *Fusarium* and mould *spp.*) and (3) the use of straw as livestock bedding (which may increase soil surface *Fusarium* inocula after farm yard manure is applied as fertiliser). In

contrast, seven of the 13 factors were described as potentially reducing the risk of mycotoxin contamination in organic farming systems compared with conventional farming systems. These included the (1) used of longer straw cereal species/varieties, (2) non-use of plant growth regulators such as chlormequat, (3) lower nitrogen input levels, (4) lower crop densities resulting from previous three factors, (5) intensive mechanical soil cultivation and tillage for weed control, (6) use of more divers crop rotations and avoidance of growing cereals after maize crops in the rotation, (7) restriction on the import of fodder components, whose effects on mycotoxin control were discussed in details in the below.

Stem length: Organic farmers are thought to use varieties with longer straw, partially because (a) this provides greater competitiveness against weeds, (b) longer straw varieties tend to have a higher protein content (which compensates for the inability to use foliar mineral nitrogen applications later in the season to increase protein content, which is common in conventional systems), (c) organic farms are more likely to have livestock and a need for straw as bedding for livestock during the winter indoor period, and (d) the risk of lodging is lower in organic farming systems, due to the non-use of mineral N-fertilisers (see section **Non-use of Mineral NPK fertilisers** below) (Almuayrifi, 2013; Rempelos *et al.*, 2018b). The initial infection of cereal plants after emergence is mainly from plant residue-borne inocula and therefore more severe if cereals are grown after maize or other cereals that are infected by the same *Fusarium* spp. (see section **More Diverse Rotations** below). However, *Fusarium* infection can also be from seed borne inoculate especially when farm saved seed are used (Limonard, 1968). *Fusarium* spores are relatively heavy and infection of younger, newly developed leaves further up the developing cereal plant is mainly via rain splash. Since the distance between leaves is greater in cereal plants with longer stems the movement of infection is thought to be slower/more difficult in longer straw varieties and the main reason for the lower incidence/severity of *Fusarium* infection and mycotoxin loads in longer straw varieties (Paulsen and Weißmann, 2002b; Burchett, 2017). In addition, infection of the grain is mainly from inoculum produced on the flag leaf and flag leaf infection is also thought to occur later in longer straw varieties.

Non-use of growth regulators and varieties with longer stems in organic systems can also reduce the infection risk in the ears because of the physically longer distance from the ground, in comparison with the short straw varieties commonly used in conventional agriculture (Paulsen and Weißmann, 2002b; Köpke *et al.*, 2007).

Non-use of mineral NPK fertilisers: Suproniene *et al.* (2012) reported that *Fusarium* grain infection levels and DON and T-2/HT-2 contamination significantly increased at higher mineral fertiliser input rates in conventional production systems using standard tillage protocols. Similar results were also reported by Heier *et al.* (2005) and Suproniene *et al.* (2012).

Gottschalk *et al.* (2007) and co-workers suggested that this was due to physiological changes associated with high levels of water soluble mineral nitrogen being available to plants in soil which is known to increase growth rates, tiller number and stem length but also thinner cell walls and lower levels of resistance-related phytochemicals (e.g. flavonoids, phenolic acids) in crops and thereby increases the susceptibility to *Fusarium* infection (Gottschalk *et al.*, 2007; Rempelos *et al.*, 2018a). At the same time, high N-availability is known to extend the vegetative growth period (and delays senescence of the flag leaf) which also extends the period over which grain infections (which are mainly from inoculum present on the flag leaf prior to senescence) can occur (Heier *et al.*, 2005; Suproniene *et al.*, 2012). In contrast, the manure and compost-based fertilisers used in organic farming system, result in a more gradual release of N and other minerals via mineralisation (breakdown of organic matter by soil) and thereby result in thicker cell walls, shorter straw and lower tillering/crop density (Al-Bataina *et al.*, 2016). However, increased use of more organic fertilisers with a high water-soluble N-content (such as chicken manure pellets, digested fish waste or blood and bone meal) that are permitted under organic farming standards may increase the susceptibility to fungal pathogens and *Fusarium* mycotoxin contamination (Köpke *et al.*, 2007). The differences in fertilisation practices (resulting in lower plant available N-concentrations in soil during vegetative crops growth stages) between organic and conventional farming systems may therefore have contributed significantly to the lower levels of *Fusarium* mycotoxin contamination found in organic compared with conventional cereals/cereal products by the meta-analyses reported here.

Lower crop density: The use of short straw varieties and especially high mineral N-inputs in conventional farming system increase tillering (Rempelos et al. 2008), crop density and humidity within the crop canopy thereby generates a more favourable microclimate for *Fusarium* heat blight (FHB) development (Al-Bataina *et al.*, 2016). In contrast, the use of longer straw varieties and lower water soluble N fertiliser in organic farming system, resulting in lower crop density, reduces the incidences of *Fusarium* diseases and *Fusarium* mycotoxins.

Mechanical cultivation: The problems associated with leaving infected plant residues on the soil surface after harvest are thought to be particularly acute with maize and in areas with high precipitation during anthesis (Dill-Macky and Jones, 2000). Incorporation of plant residues using an inverting plough in organic farming system is known to be the most efficient tillage method for reducing levels of *Fusarium* inoculum on the soil surface, the incidence of FHB and DON levels in wheat, especially when wheat is grown after maize. Although traffic and tillage may cause problems with subsoil compaction in organic agriculture (Schjønning *et al.*, 2002), direct seeding and reduced tillage systems are currently rarely used, owing to the problems of controlling weeds in systems that do not permit herbicides. However, no/minimum

tillage is increasingly used in conventional agriculture, resulting in higher incidences of *Fusarium* diseases and *Fusarium* mycotoxin levels (Krebs *et al.*, 2000).

More diverse rotations: Compared with conventional crop rotations, organic crop rotations are often more diverse, and include a higher proportion of rotational grassland or forage crops and more nitrogen-fixing crops including grain legumes such as soybean, lentils and peas, and forage legumes such as clover and vetch (Barbieri *et al.*, 2017). With 0.7 years longer and 48% more crop categories, organic rotations contribute to improving soil organic matter, biodiversity and biological activity, especially when used in combination with regular organic matter/fertilizer inputs (Mader *et al.*, 2002; Alabouvette *et al.*, 2004; Barbieri *et al.*, 2017). While maize as a pre-crop was shown to substantially (up to 100) increase *Fusarium* head blight and mycotoxin concentrations ,soybean or grass as pre crops reduced mycotoxin levels in wheat when compared with wheat grown after a wheat pre-crop (Dill-Macky and Jones, 2000; Birzele *et al.*, 2002b). This is thought to be mainly linked to the higher levels of *Fusarium* inoculum present on crop residues after pre-crops such as maize and wheat (Dill-Macky and Jones, 2000). The *Fusarium* mycotoxin risk after pre-crops such as maize and wheat was reported to further increase when used in combination with no or minimum tillage, which is common in conventional, but rarely used in organic farming systems.

Restriction on the import of fodder components: In organic system, diversified rotations with a lower proportion of cereals and a higher percentage of fodder crops, in combination with regular organic matter-based fertility inputs, were also linked to soil with (1) higher contents and turnover of soil organic matter and (2) great soil biodiversity and biological activity. These characteristics are also thought to increase the suppressive nature of soil and thereby reduce pathogen inoculate (Alabouvette, 1990; Knudsen *et al.*, 1995; Krebs *et al.*, 2000; Alabouvette *et al.*, 2004).

Non-use of strobilorin fungicides: There are also studies which showed that the use of strobilurin fungicides will increase the risk of *Fusarium* mycotoxin contamination in conventional cereal grains/products (Müllenborn *et al.*, 2008).

It is important to point out in this context, that significantly lower DON contamination in organic crops were detected in regions/countries (e.g. Austria, Germany, and Belgium) with environmental conditions (Dfb and/or Cfb) that are known to result in a relatively high risk of *Fusarium* mycotoxin contamination (Table 2.10). However, large-scale farm surveys of the UK and Norway, which are thought to have a low risk of *Fusarium* head blight and mycotoxin contamination, also reported higher *Fusarium* contamination in conventional cereal grains (Edwards, 2009b; Edwards, 2009a; Bernhoft *et al.*, 2012).

However, it should also be noted, that the use of fungicide seed treatments and foliar application of fungicides (those shown to reduce *Fusarium* infection/mycotoxin risk) is an important risk mitigation method in conventional cereal production, given that many of the preventative agronomic practice are less widely used (Paulsen and Weißmann (2002a). Also, until efficient acceptable seed treatments are available, seed-borne *Fusarium* inocula remain a risk factor in organic systems, especially where farm-saved seed is used (Kadege and Lyimo, 2015).

The finding for considerable confounding effects of cereal species, climatic conditions and country was expected, and is consistent with previous studies showing (a) significant differences in the susceptibility of different cereal genera, species and varieties to *Fusarium* infection and mycotoxin grain contamination (Gilbert and Tekauz, 2000; Rudd *et al.*, 2001; Foroud and Eudes, 2009) and (b) that climatic conditions during the growing period are a major factor determining the frequency of occurrence and concentrations of *Fusarium* mycotoxins in cereal grains (Hope *et al.*, 2005; Köpke *et al.*, 2007; Paterson and Lima, 2010; Van Der Fels-Klerx *et al.*, 2012).

For example, high incidences of *Fusarium* infection in common wheat grain have frequently been linked to high rainfall and long periods of leaf wetness/high humidity during the vegetative growth stage and especially the flowering stage of cereals (Birzele *et al.*, 2000; Birzele *et al.*, 2002a). Climatic conditions favouring lodging of cereals (strong winds and leaf wetness at later growth stages) will also increase the risk of infection and mycotoxin production from both *Fusarium* spp. (Easson *et al.*, 1993; Nakajima *et al.*, 2008).

Resistance to fungal infection by mycotoxin producing *Fusarium* and mould species is known to differ between cereal genera (maize, wheat, barley, oats, rye), species (e.g. common vs spelt wheat) and varieties/genotypes (Foroud and Eudes, 2009). For example, common wheat was reported to be more frequently contaminated with DON than rye (Tanaka *et al.*, 1988; Döll *et al.*, 2000), while DON accumulation in oats was more severe than in common wheat (Foroud and Eudes, 2009). Those differences were explained by diverse *Fusarium* heat blight (FHB) resistance of cereals (Foroud and Eudes, 2009). This might explain the results of the meta-

analyses based on the multi-level random-effects model which identified differences in (a) DON contamination between organic and conventional samples for common wheat and rye, but not oats, rice and barley and (b) OTA contamination for rice, rye and barley, but not common wheat and oats.

2.5.2 Mould mycotoxins

Different to the *Fusarium* mycotoxins, contamination with mould mycotoxins such as OTA has often been linked to insufficient post-harvest drying of grain and poor grain storage conditions (Magan and Aldred, 2005; Magan and Aldred, 2007; Magan *et al.*, 2010; HGCA, 2014). Insufficient drying and poor storage conditions may also exacerbate of concentrations of DON and other *Fusarium* mycotoxins already present at harvest (Magan *et al.*, 2010).

Studies by Magan and Aldred (2007) and Magan *et al.* (2003) indicated that water availability and temperature are the most important abiotic factors influencing growth and OTA production by spoilage fungi. According to current recommendations the target safe moisture content is 14-14.5% for common wheat, for barley and oats it is 14-14.5%, and it is 13-14% for rice (Magan and Aldred, 2007). These thresholds have been set because *Fusarium* spp., *Pennicillium spp*., and *Aspergillus spp.* need at least 17-19% humidity to grow on cereal grains (Magan *et al.*, 2002; Cairns-Fuller *et al.*, 2005; Magan and Aldred, 2007; Magan *et al.*, 2010). The storage temperature after harvest has an effect on fungal growth too, especially in silo storage where temperature control is critical. In particular, a good ventilation that incorporates cooling and drying operations is necessary to avoid enhanced mycotoxins contamination during storage (Jouany, 2007; Nesic *et al.*, 2014).

Lodging of cereals will also increase the risk of infection and mycotoxin production from both *Fusarium* spp. and common moulds (Easson *et al.*, 1993; Nakajima *et al.*, 2008).

Pre-harvest use of fungicide is therefore not an effective approach for controlling common mould mycotoxins in cereals and postharvest fungicide applications are prohibited in Europe.

The importance of efficient postharvest drying and storage for minimising OTA contamination may also explain the finding that OTA contamination was substantially higher in organic than conventional cereal grains/products especially prior to 2004. At this time, average concentrations in organic cereal grains/products were 3.1 µg/kg thus exceeding the current maximum levels set by the EC for OTA and several comparative studies from that period concluded that the higher OTA contamination in organic grains was due to poor grain drying and/or storage facilities available on organic farms (Jorgensen and Jacobsen, 2002; Köpke *et al.*, 2007).

The finding that OTA contamination decreased over time and was very similar in the last time period (2010-2015) analysed in this study may therefore at least partially been due to improvements in grain drying and storage facilities in the organic sector over the last 15 years, However, the introduction of improved mycotoxin testing based quality assurance systems may also have contributed to the reduction on OTA levels in organic cereals over time.

OTA is nephrotoxic, immunosuppressive, carcinogenic and teratogenic in all experimental animals tested (Bui-Klimke and Wu, 2015) . The relatively low thresholds (0.5 µg/kg for infants/young children and 3 µg/kg in older children and adults) set for OTA by the European Commission (European Commission, 2006a) reflect the considerable health risks and especially cancer-promoting activity that were linked to even very small intakes of OTA (Tables 2.5 and 2.6) (Peraica *et al.*, 1999a; Walker, 2002; Bhat *et al.*, 2010; Reddy *et al.*, 2010; Bui-Klimke and Wu, 2015). A substantial amount of cereals are rejected for use in human consumption due to high OTA levels and it is therefore important to investigate how OTA levels can be further reduced (e.g. through (a) crop breeding (b) innovations in primary production, especially harvest and post-harvest grain management practices and (c) quality assurance procedures in grain storage/processing facilities).

2.5.3 Changes in levels of mycotoxin contamination over time

Results indicate that contamination did not only decrease for (a) OTA in organic cereal grains/products, but also (b) ZEA in conventional, and (c) total fumonisins, T-2/HT-2 mycotoxins and aflatoxins in both organic and conventional cereal grains/products (Figure 2.2, Appendix 2.4). Since most comparative studies were carried out in Europe, the most likely explanation for the decrease in contamination in both organic and conventional cereal grains/products is that grain storage, marketing and processing companies have made substantial improvements to their post-harvest quality assurance protocols after legal maximum contamination levels were introduced by the (European Commission, 2006a). Previous studies

Specifically trends towards a decrease in the concentrations of some mycotoxins have been linked to the development of (a) regulatory systems and testing regimes for mycotoxins (European Commission, 2006a; van Egmond *et al.*, 2007) and/or (b) improved agricultural and post-harvest processing/storage practices and/or (c) application of improved HACCP-systems throughout the grain supply chain (Aldred *et al.*, 2004; Kabak *et al.*, 2006).

Climate change may also have contributed, since climatic conditions are known to be the most important factor affecting of mycotoxin contamination in cereal grains especially during later cereal growth stages and at harvest (Langseth and Elen, 1997; Birzele *et al.*, 2002b). A recent review by Paterson and Lima (2010) discussed potential effects of climate change on

mycotoxin contamination risks. It concluded that areas which become dryer and hotter during the growing season mycotoxin contamination may decrease, while colder regions which become temperate and wet will experience greater problems associated with both *Fusarium* and mould mycotoxins. For example, an increase of temperatures in cold countries like Norway may therefore cause an increase in both *F. graminearum* and *F. langsethiae* infections resulting in higher levels of DON and HT-2/T-2 contamination, respectively (Bernhoft *et al.*, 2012). Changes in climatic conditions would be expected to have the same principal effect on both organic and conventional farming systems (Paulsen and Weißmann, 2002a).

However, it should be pointed out that primary production protocols of both organic and conventional cereals have changed relatively little over the last 20 years, and are therefore unlikely to have contributed significantly to reducing mycotoxin contamination. Also, some agronomic practices that increase mycotoxin risk (e.g. minimum tillage in conventional farming and growing wheat after wheat or maize pre-crops in organic production) are thought to be more widely used now than 20 years ago (Qiu *et al.*, 2016; Townsend *et al.*, 2016; Riley, 2017). This may indicate that most of the improvements in mycotoxin loads were due to improvements in postharvest treatments and quality assurance procedures and future studies should therefore investigate whether this has resulted in an increase in the (a) the mycotoxin loads in cereals used for animal production and/or animal products (e.g. milk) and (b) the proportion of cereals that needed to be discarded because they were unsuitable for both human and animal consumption.

2.6 Conclusions

Overall, the results of the meta-analysis reported here suggest that historically conventional cereals had higher levels of *Fusarium* mycotoxin contamination, while organic cereals had higher level of OTA contamination. Results, also suggest that average mycotoxin loads have decreased over time and are now (a) are broadly similar in organic and conventional cereals and (b) substantially lower than the maximum levels set by the EC for grains/products destined for adult human consumption.

This indicates that improvements post-harvest drying, storage, and quality assurance facilities/protocols (e.g. introduction of detailed mycotoxin testing) since the early 2000s has resulted in significant reductions in mycotoxins entering the human food chain.

Future research should therefore focus on improving agronomic protocols and genetic resistance against mycotoxin producing *Fusarium* and mould fungi, especially in regions for which climate change is predicted to increase mycotoxin pressure.

One particular area of concern is that, average concentrations of OTA in both organic and conventional cereal grains/products in the last time period examined (2010 to 2015) were still two times higher than the maximum levels (0.5 µg OTA per kg) set by the EC for cereal-based foods and baby foods for infants and young children.

Also, the risk of exposure to mixtures of mycotoxins has rarely been investigated and exposure to mixtures of mycotoxins may still affect human and animal health, even if concentrations for each individual mycotoxin are below the EC threshold. The magnitude of relative difference overall *Fusarium* mycotoxin intake levels via a switch to organic cereal consumption are also currently impossible to estimate. There are, to our knowledge, no human epidemiologica/cohort and/or dietary intervention studies in which health impacts of contrasting mycotoxin intakes with conventional and organic foods were investigated.

As expected, the multilevel meta-analysis model identified cereal species as major confounding factors for the comparison of mycotoxin contamination in organic and conventional cereal grains/products, but there was insufficient information in the primary publications to include other potential confounding factors (e.g. cereal variety choice, use of irrigation and irrigation method, use of farm-saved seed, and/or whether whole meal or white flour based cereal products were compared). These gaps in knowledge should also be investigated in future studies.

Chapter 3 General Materials and Methods

The nutritional components analysed in the flour samples collected for the shopping basket study (SBS) and during the field experiments (EX), included dry matter, crude protein, phenolic phytochemicals, micro and macro minerals, mycotoxins and pesticides. The materials and methods for those analyses are described below:

3.1 Dry matter

All samples were dried in an oven at 130^0C for 2 hours and allowed to cool down for one hour in desiccator cabinets before weighing to calculate dry matter for expressing results.

3.2 Crude protein content analysis

Crude protein is usually estimated by multiplying nitrogen (N) content by a nitrogen to protein conversion factor. In most foods, including flour, amino-N accounts for approximately 16% of the protein weight. Hence, the nitrogen to protein conversion factor is usually 6.25 (Simonne *et al.*, 1997).

Grain N concentration of flour samples was determined by the total combustion method using a vario MACRO cube C/N Analyzer (Elementar LTD, Germany). Around 50 mg of fresh sample was weighed into a tin foil cup. The cup was carefully folded and squashed into a pellet to expel the air using a tool provided by Elementar. Before each run, a set of control standards were run to ensure that the analyser was working correctly. The N results were multiplied by 6.25 to estimate grain crude protein concentration.

3.3 Phenolic phytochemical content and antioxidant capacity analysis

Various assays purporting to measure phenolic phytochemical content and antioxidant capacity have been reported and they have pros and cons. In this project, Folin-Ciocalteu assay (Singleton *et al.*, 1999; Zhang *et al.*, 2006), Trolox Equivalent Antioxidant Capacity (TEAC) assay (Re *et al.*, 1999b), Ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996), and aluminum chloride colorimetric method (Zhuang *et al.*, 1992; Liu *et al.*, 2002) were used with some modifications to measure (a) total phenolic content, (b) total antioxidant capacity (TEAC and FRAP) and total flavonoid content of flour samples. In addition, to investigate the complete phenolic phytochemical profiles that exist in flour samples, the free, insoluble bound and soluble conjugate forms were assessed.

3.3.1 Extraction

The phenolic phytochemicals were extracted from the flour samples into three separate fractions: soluble free, soluble conjugated, and bound using the method described by Adom and Liu (2002) with some modifications. All extractions for each sample were repeated in triplicate.

Soluble Free Fraction: 0.025g of sample was mixed with 1 mL of 80% chilled ethanol for 10 mins with continuous shaking at room temperature followed by sonication in a sonic bath for a further 6 mins. After centrifugation at 13200 rpm for 5 mins, the supernatant was removed and extraction was repeated twice. Supernatants were combined and then evaporated under nitrogen gas flow at 35 ^{0}C to dryness and reconstituted in 250 µL of ultrapure water. The extracts were stored at -80^{0}C until use.

Bound Fraction: 0.01g of sample was mixed with 1mL of 80% chilled ethanol for 10 mins with continuous shaking at room temperature followed by sonication in a sonic bath for a further 6 mins. After centrifugation at 13200 rpm for 5 mins, the supernatant was removed for conjugate fraction extraction (see below). The remaining residue was then digested with 800 µL 2M sodium hydroxide at room temperature for 4 hours with 1 min shaking each half hour. The mixture was neutralized with 120 µL hydrochloric acid (HCl) and the solution was extracted three times with 800 µL ethyl acetate. The ethyl acetate fraction was evaporated under nitrogen gas flow at 35 ^{0}C to dryness. Phenolic compounds were reconstituted in 250 µL water of water and stored at -80 ^{0}C until use.

Soluble Conjugated Fraction: Ethanol extracts from the bound phenolic extractions above were used for soluble conjugated extractions. The extracts were dried under nitrogen flow at 35 ^{0}C and then were digested with 400 µL 2M NaOH for 4 hours, and the solution was neutralized with 80 µL HCL. The mixture was extracted three times with 500 µL ethyl acetate, and the ethyl acetate fraction was evaporated to dryness at 35 ^{0}C under nitrogen gas flow. Phenolics were recovered for analysis in 250 µL water and stored at -80 ^{0}C until use.

3.3.2 Total phenolic phytochemical content analysis

The total phenolic phytochemical content of wheat extracts was quantified using the Folin-Ciocalteu method (Singleton *et al.*, 1999; Zhang *et al.*, 2006) with minor modifications. Standard solutions of gallic acid were prepared as follows: 20 g gallic acid were dissolved in 3 mL methanol then made up to volume with distilled water in a 100mL flask. The standards were serially diluted to create a standard calibration curve. The concentration of standards for serial dilutions were 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL and 3.125 µg/ml.

20 µL of each sample solution, the serial standard solutions and distilled water as blank were added to wells on a 96-well microplate. Each standard solution and sample solution was run in duplicate. 130 µL of Folin-Ciocalteu reagent (diluted by distilled water 1:10 (v/v)), was added to each well. After 5 min 100 µL of 7.5% sodium carbonate (Na_2CO_3) solution was added. The plate was covered with a plastic cover and incubated in the dark at 40^0C for 30 minutes. The absorbance of all solutions was measured at 760nm with a spectrophotometric microplate reader (Konica Minolta, Tokyo). Final results were presented as µmol gallic acid equivalent (GAE)/g flour (DW).

3.3.3 Analysis of Total Antioxidant capacity by TEAC and FRAP

Total antioxidant capacity of wheat extracts was measured by both TEAC and FRAP methods (Benzie and Strain, 1996; Re *et al.*, 1999a).

Trolox (6-hydroxy-2,5,7,8-tet-ramethychroman-2-carboxylic acid) was used as an antioxidant standard for the **TEAC assay**. 6.3mg Trolox were dissolved in 50 mL flask using 50% methanol, then were serially diluted by distilled water to concentrations of 126, 63, 31.5, 15.75, 7.875, 3.9375 and 1.96875 µg/mL for creating a standard calibration curve. **ABTS working solution preparation**: Solution A, 2.45 mM potassium persulfate ($K_2S_2O_8$) was obtained by dissolving 66.2 mg $K_2S_2O_8$ (Sigma, Poole, Missouri) in 100 mL distilled water. Solution B, 7 mM ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) was prepared by dissolving 192 mg in 50 ml of distilled water. Solutions A and B were mixed in proportion of 1:9 (v/v) and left in the dark at room temperature overnight to generate the ABTS working solution. Prior to use, ABTS working solution was diluted and adjusted to an absorbance of 0.7±0.02 at λ 760nm using 5 mM phosphate buffer solution (pH=7.4), which was prepared by mixing 4.5g sodium chloride (NaCl), 0.1839g sodium phosphate monobasic ($NaH_2PO_4 \cdot H_2O$) and 0.3677g sodium phosphate dibasic dodecahydrate ($Na_2HPO_4 \cdot 12H2O$) in 500 mL of distilled water. **Analysis**: 10 µL of Trolox standards, samples and 50% methanol with distilled water as blank were mixed with290 µL of TEAC working solution in a 96-well microplate. The decrease in absorbance was measured at 734nm after 6 min incubation at 37 ^{0}C. Each standard solution and sample solution was run in duplicate. The final results were expressed as µmol Trolox equivalent (TE)/g flour (DW).

For the **FRAP assay**, 0.278g Ferrous Sulphate ($FeSO_4.7H_2O$) were dissolved in 1L distilled water is as the standard stock solution. This was diluted to 278, 139, 69.5, 34.75, 17.375, 8.6875 and 4.34375 µg/mL by serial dilution for the standard calibration curve. **FRAP working solution preparation**: Solution A: acetate buffer (pH 3.6) was prepared by dissolving 3.1g sodium acetate trihydrate ($CH_3COONa_3H_2O$) in a 100 mL flask using about 50 mL distilled water, this was mixed gently with16 mL concentrated acetic acid (CH_3COOH), then made up

to volume with distilled water. Solution B: 10mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ, $C_{18}H_{12}N_6$), was prepared by dissolving 0.0781 TPTZ I in 25 mL of 40mM HCl. Solution C: 20mM ferric chloride ($FeCl_3.6H_2O$), was prepared by dissolving 0.5406 g $FeCl_3.6H_2O$ in 100 mL H_2O. The FRAP working solution was prepared by mixing solution A, B, C in proportion of 10:1:1. Fresh FRAP working solution was prepared before each assay. **Analysis**: 10µL of ferrous sulphate standards, extraction samples and distilled water as blank were mixed with 300µL of FRAP working reagent in the 96-well microplate and incubated at 37^0C for 4 minutes. The absorbance of samples was measured at λ 593nm after incubation. Each standard solution and sample solution was run in duplicate. The final results were expressed as µmol Fe^{2+} equivalent/g flour (DW).

3.3.4 Analysis of Total Flavonoid content

The total phenolic content of wheat extracts was determined by a colorimetric method described previously (Liu *et al.*, 2002) with minor modification. 15 g catechin were dissolved in a 100mL flask with 10 mL methanol then made up to volume with distilled water. The standards were serially diluted to create a standard calibration curve. The concentration of standard from serial dilutions were 150 µg/mL, 75 µg/mL, 37.5 µg/mL, 18.75 µg/mL, 9.375 µg/mL, 4.6875 µg/mL and 2.34375 µg/mL.

25 µL of each sample solution, the serial standard solutions and distilled water as blank were loaded on a 96-well microplate. Each standard solution and sample solution was run in duplicated. 7.5 µL of a 5% $NaNO_2$ solution was added to all sample solutions, standards and blanks. After 6 min at room temperature, 15 µL of 10% $AlCl_3.6H_2O$ solution was added and the mixture was allowed to stand for another 5 min at room temperature. Then 50 µL of 1 M NaOH were added to the mixture followed by 152.5 µL distilled water. The absorbance was measured immediately at λ 510nm using the spectrophotometric microplate reader (Konica Minolta). The results were expressed as µmol catechin equivalent (CE)/g flour (DW).

3.4 Phenolic Profile analysis by HPLC

The phenolic phytochemical profile in the flour samples was investigated by a high-performance liquid chromatography (HPLC) method.

Extraction of samples for phenolics profile analysis by HPLC was as described in section 3.3.1. All chemicals were purchased from Sigma-Aldrich. Phenolic phytochemical standards (4-hydroxyvalproic acid, vanillic acid, syringic acid, p-coumaric acid, syringaldeyde, sinapic acid, ferulic acid) were prepared as a stock solution at 0.1 mg/mL in 70:30 methanol:water, and were stored at -20 ^{0}C in the dark until HPLC analysis was performed, within three months of extraction.

Flour extracts were analysed by HPLC on a Shimadzu Prominence HPLC system equipped with an LC-20AD pump, SIL-20AC antosampler, and SPD-M20A photodiode array detector (Shimadzu Crop., Kyoto, Japan). Data collection and integration were performed using LabSolution software. Phenolic acids were separated on a reverse-phase Thermo Scientific Hypersil C18 column (250 × 4.6mm, 5µm particle size). The column was heated at 25 ^{0}C while the samples tray temperature was set to 4 ^{0}C.

Mobile phase A was acetonitrile, while mobile phase B was 0.1% acetic acid. The gradient programme for the mobile phase (A:B) was at 0.02 min (5:95), 10 min (20:80), 15 min (25:75), 20 min (35: 65), 25 min (65: 36), 25.01 (100:0), 30 min (100:0), 30.01 (5:95) and 40 (5:95). The flow rate of the mobile phase was 2 mL/m, and the injection volume was 20 µl. Scanning was performed from 190 nm to 800 nm, and phenolic acids were identified by comparing retention times and UV-VIS spectra with those of pure standards. Concentrations, expressed in µg/g DW, were calculated at 230, 270 or 320 nm using calibration curves of phenolic acid standards. The following phenolic acid peaks were identified and quantified according to their spectra and relative retention time: 4-hydroxyvalproic acid, vanillic acid, syringic acid, p-coumaric acid, syringaldeyde, sinapic acid, ferulic acid.

3.5 Analysis of Macro and micro mineral nutrients

The inductively coupled plasma (ICP) optical emission spectroscopy (OES) technique (ICP-OES) was used to analyse macro and micro mineral nutrients in this project and it is a typical multi-element detection method and permits the fast and reliable simultaneous determination of whole range of these inorganic species. For sample preparation, microwave acid digestion was used by exposing samples to a strong acid in a closed vessel and raising the temperature and pressure through microwave irradiation to dissolve metals bound within the flour matrix.

3.5.1 Digestion

0.25g flour was mixed with 5 mL 69% nitric acid (HNO_3) in Teflon vessels then digested in a microwave reaction unit (CEM-Mars 6, USA) in “vegetable” mode with a four step heating program (step 1 ramp to 180 ^{0}C; step 2 hold 180 ^{0}C for 10 minutes; step 3 ramp to 205 ^{0}C for 20 mins; step 4 cooling down) . After digestion, samples were allowed to cool to room temperature and then were filtered through blue ribbon quantitative filter paper (Whatman Grade 589/3), the filtrates were mixed with distilled water and diluted with ultrapure water in 50 mL flasks. Digested solutions were stored in Sterilin tubes at 4 ^{0}C until analysis.

3.5.2 Analysis by ICP

Macro and micro minerals in digested solutions were analysed with Inductively Coupled argon Plasma Optical Emission Spectrometer (ICP-OES) equipped with a CCD detector (Vista-Pro Axial; Varian Pty Ltd., Mulgrave, Australia). Analytical quality was checked against the certified values of the quality reference material (wheat flour SRM 1567a and apple leaves SRM1515) which were included in every batch of 40 samples.

3.6 Analysis of grain Mycotoxin content

An enzyme-linked immune-sorbent assay (ELISA) method was used in this project to analysis the mycotoxin content in flour samples, which was based on the ability of a specific antibody to distinguish the three-dimensional structure of a specific mycotoxin. Due to the high cost of HPLC, HPLC-MS or GC-MS based analyses and the large number of samples that needed to be examined to identify potentially confounding effects of country, flour type and wheat species we used the commercial ELISA-based test kits in this study, which reduces the incubation time to minutes. Whilst the method is faster it is less sensitive and accurate than MS-based methodologies but is regularly used in the milling industry to screen grain samples for contamination and was therefore considered appropriate for this study. In this study, the most important mycotoxins DON, ZEA and T-2/HT-2 produced by *Fusarium spp* and the storage mycotoxin OTA produced by *Penicillium*, *Aspergillus* spp. were analysed.

Mycotoxin assessment was carried out in the lab of Coastal Grains Ltd, Belford, UK (Northumberland's largest grain co-operative). All samples were analysed using ROSA (Rapid One Step Assay) CharmScience Test Kits, standard industry tests used in commercial mills. The following strips were used to test for levels of specific mycotoxins: ROSA FAST5 DON Quantitative Test; ROSA ZEARQ-FAST5 Zearalenone Quantitative Test; ROSA Ochratoxin A Quantitative Test for Feed and Grain, and ROSA T-2/HT-2 Quantitative Test. The ROSA Charm Science Test kit is a quantitative lateral flow test, read in the ROSA-M Reader. All kits were stored at 4°C and Controls in kits were used to ensure that the reader was working correctly. During the analysis progress, all steps followed the procedures indicated by Charm Science.

Deoxynivalenol (DON): 10g flour samples were weighed and mixed with 50 mL distilled water for 30s with continuous shaking at room temperature. After centrifuging for 20 seconds, 100 µL of extract was diluted with 1 mL buffer and mixed well. 300 µL of the diluted extract was pipetted into the sample compartment. The strip was incubated for 5 min then read in the ROSA-M reader using the DON channel.

Ochratoxin A (OTA): 10g flour samples were weighed and mixed with 20 mL 70% Methanol for 1-2 min with continuous shaking at room temperature. After centrifugation for 20 seconds, 100 µL of extract was diluted with 1 mL buffer and mixed well. After centrifugation for 2 min, 300 µL extract was pipetted into the sample compartment. The strip was incubated for 10 min then read in the ROSA-M reader using the ORCH channel.

Zearalenone (ZON) and **T-2/HT-2 toxin**: 10g flour samples were weighed and mixed with 20 mL 70% Methanol for 1-2 min with continuous shaking at room temperature. After centrifugation for 30 seconds, 100 µL of extract was diluted with 1 mL buffer then mixed well. After filtration, 300 µL of extract was pipetted into the sample compartment. The strip was incubated for 5 min then read in the ROSA-M reader using the ZON/T-2 channel.

Since the ROSA reader can only be calibrated by professional operators in the original product factory, a quality assessment was performed before the analysis was carried out in the lab. The coefficient of variation for the quality assessment of DON, OTA, T-2/HT-2 and ZEA mycotoxins were 15%, 20%, 19% and 15% respectively for the control sample, and 18%, 23% 31% and 29% respectively for a random unknown flour sample.

3.7 Analysis of grain pesticide residues

Pesticide residue analyses were carried out by the Benaki Phytopathological Institute (Stefanou Delta Street, Kifissia, Athens, 14561) in 2016 and Concept Life Sciences Ltd. (19 Spring Gardens, Manchester, UK; www.conceptlifesciences.com) in 2017.

In both laboratories GC-ECD, GC/MS and LCMSMS were used for identification and quantification of the pesticides and their metabolites in flour, using validated analytical methods. Due to the contrasting physicochemical properties of the pesticides, 4 different extraction methods were used for (1) multi-residues; (2) 2.4D and fluazifop; (3) chlormequat and mepiquat; and (4) glyphosate, respectively, and 3 different analyses methods (GC-ECD, GC/MS and LCMSMS) were applied. Most of the analyses were extracted and determined with a multi-residue method as described below, except in the cases of 2.4D, fluazifop, chlormequat, and mepiquat.

Multi-residue method: For the extraction of the majority of the analyses, the protocol of the QuEChERS method concerning commodities with high fat content was followed as described previously (Anagnostopoulos and Miliadis, 2009; Anagnostopoulos *et al.*, 2010). The LC-MS/MS analysis was performed by an Agilent Series 1200 liquid chromatograph equipped with a reverse phase Zorbax Eclipse XDB C18 3.5µm particle size, 150mm x 2.1mm analytical column (Varian, Palo Alto, CA, USA). Detection was achieved using a triple quadrupole mass spectrometer (Agilent Triple Quad 6410) equipped with an electrospray ionization interface

operating in positive mode. For the GC analysis, pesticides were separated and determined in two Agilent 6890 gas chromatographs, with a splitless injectors equipped with a DB-5-MS column (30 m, 0.32mm i.d. and 0.25 μm film thickness) and a DB-17 MS column (30 m, 0.3mm i.d. and 0.25 μm film thickness) each connected to an ECD detector.

Analysis of 2.4D and fluazifop: For the extraction of 2.4D and fluazifop a different variation of the QuEChERS method for acidic pesticides was adopted (European Commission, 2007). In this variation, before addition of acetonitrile, 300μL of 5N NaOH solution were added to adjust the pH to 12. The tube was shaken vigorously for 1min and the mixture was left to stand for 30min. Then 300μL of 5N H_2SO_4 solution and acetonitrile was added. Detection was performed with LCMSMS (Anagnostopoulos *et al.*, 2013).

Analysis of chlormequat, mepiquat: For the extraction of chlormequat and mepiquat, the QuPPe protocol was used (Anastassiades *et al.*, 2016) and the determination was conducted using an LC-MS/MS (Danezis *et al.*, 2016).

Analysis of glyphosate: Similar for the extraction of glyphosate, the QuPPe protocol was used (Anastassiades *et al.*, 2016) in combination with FMOC derivatization as follows: An aliquot of 5 ± 0.05 g was weighted in a 50 mL centrifuge tube and an appropriate amount of HPLC water was added in a ratio ranging from 1:2 to 1:3, depending on the product. The homogenization time depended on the size and nature of the sample. The sample slurry was extracted with 10 mL of methanol. The mixture was shaken using an orbital shaker for 2 hours (in case of a big batch of samples) or using an Ultra Turrax (T25 Basic Ultra Turrax) for 3 min. (in case of individual samples) and centrifuged at 4000 rpm for 5 min. An aliquot of 5 mL was transferred into a 15 mL plastic centrifuge tube and let in the freezer for at least 2 hours or overnight. For the derivatization, an aliquot of 2 mL was transferred into a 15 mL plastic centrifuge tube and 1 mL borate buffer (pH=9) and 0.5 mL of 6000 ppm FMOC solution was allowed to react for 10min. at 70^{o}C. Before injecting in the chromatographic system, the final solution was filtered through a 0.22 μm disposable Cellulose syringe filter. The LC-MS/MS analysis was performed by an Agilent Series 1200 liquid chromatograph equipped with a reverse phase Zorbax Eclipse XDB C_{18} 3.5μm particle size, 150mm x 2.1mm analytical column (Varian, Palo Alto, CA, USA). Detection was achieved using a triple quadrupole mass spectrometer (Agilent Triple Quad 6410) equipped with an electrospray ionization interface operating in negative mode. The identification of glyphosate was based on retention time (R.T.= 15.4) and the presence of coinciding peaks for two selective transitions (m/z 390 → 168 and 390 → 160) in the correct abundance ratio.

3.8 Statistical analysis

Analyses of variance (ANOVA) were derived from non-linear mixed-effects models (Pinheiro and Bates, 2000). The analyses were carried out using the nlme function in the nlme package in the R statistical environment (R Develoment Core Team, 2012) and residual normality was assessed using the qqnorm function in R. The interactions between factors were tested by using Tukey contrasts in the general linear hypothesis testing (glht) function of the multcomp package in R. The means and standard errors where calculated using Minitab Software Version 17.

Chapter 4 Quality of organic and conventional Spelt wheat and common wheat flour: A shopping basket survey in UK and Germany

4.1 Introduction

Cereal products are the most important source of nutrients and energy in the human diet, and wheat is one of the most important cereal species consumed globally (McKevith, 2004).

Spelt wheat (*Triticum spelta*), one of the most ancient wheat species, is currently increasing its share of the cereal market (Escarnot *et al.*, 2012). This is thought to be mainly because of its ability to grow under low input conditions (which make it particularly suitable for organic farming systems) and consumer perceptions that minor cereals including spelt wheat have a nutritional advantages compared with common wheat (*Triticum aestivum*) (Dean *et al.*, 2007). However, due to a lack of scientifically sound comparative studies there is still considerable uncertainty about whether or not and to what extent spelt wheat has a superior nutritional composition compared with common wheat. There have been several studies in which the nutritional composition of common wheat and/or spelt wheat grains or food products made from them were analysed (Rüegger and Winzeler, 1993; Escarnot *et al.*, 2012; Calzuola *et al.*, 2013). However, to our knowledge, there have been no comparative shopping basket study (SBS) in which (a) the composition of matching common and spelt wheat based food samples collected from the same retail outlets were compared and (b) confounding factors such as sample region (e.g. different countries), farming system (e.g. organic vs conventional) and grain processing method (e.g. white or wholemeal products) were considered in the survey design.

Wheat flour, is the main ingredient in many staple food products such as breakfast cereals, pasta, noodles, bread and other bakery products and has been one of the major constituents of the human diet for several thousand years (Goesaert *et al.*, 2005). Currently the majority of wheat products are based on white flour (where the bran is removed and flour is made from the endosperm). However, the use of wholemeal flour (where the whole grain is present in the flour after milling), is increasingly recommend by nutritionists in recent decades because an increasing number of scientific studies were shown associations between wholemeal consumption and a reduced risk of chronic disease such as cardiovascular disease, type 2 diabetes and obesity (Jones and Engleson, 2010; Cho *et al.*, 2013). The flour supplied in the markets for customers are good sources for investing in the nutritional quality and the potential risk of health food brings with. The health benefits from wholemeal consumption are thought to be mainly associated with the higher fibre, mineral and (poly)phenol/antioxidant content (which is mainly in the bran fraction of the grain) of wholemeal flour (Jones and Engleson, 2010). However, there are also some studies which reported the presence of higher concentrations of pesticides and mycotoxins in the bran fraction (which is removed when white flour is produced) (Weidenbörner *et al.*, 2000; Edwards *et al.*, 2011; Vidal *et al.*, 2013). In addition, concentrations of pesticide would also be expected to be higher in

the outer layers/bran fraction of the grain (Bordin *et al.*, 2017). If these were confirmed, this would be considered nutritionally undesirable by many consumers (Lee and Yun, 2015). Organic production methods were recently shown to result in higher antioxidants/phenolics and Zn, but lower Cd and pesticide content in cereals/cereal products including wheat grains (Cooper et al. 2013; Baranski et al. 2014; Rempelos et al. 2018). It is therefore important to consider the potential confounding effects of cereal production methods in studies comparing the mineral, phytochemical and toxic metal content of white and whole meal cereal products available to the consumer.

4.2 Aims

The **overall aim** of the study was to obtain a more accurate estimate of the nutritional differences and undesirable components' differences between spelt and common wheat grain by analysing all brands of wheat flour that were assessable in Germany and the UK (thereby estimating differences between the common and spelt wheat varieties currently used), while accounting for the confounding effects of production system and flour type (white vs wholegrain).

The **main objectives** of this study were therefore (a) to compare antioxidant capacity, and protein, phenolic phytochemical and mineral concentrations in white and wholemeal common and spelt wheat flour brands/products available in the UK and Germany (b) to study the effect of primary production methods organic and conventional protocols on antioxidant capacity, and protein, phenolic phytochemical and mineral micronutrient concentrations in wheat flour and (c) identify potential interactions between primary production protocols, wheat species and post-harvest processing with respect to antioxidant capacity, and protein, phenolic phytochemical and mineral micronutrient concentrations.

The minerals assessed included all plant macro- and micronutrients, the undesirable elements aluminium and nickel and the toxic metal cadmium.

4.3 Shopping basket study experimental design

The SBS of wheat flour was conducted over three successive years in 2015, 2016 and 2017. The experimental design included 3 factors/variables: wheat species (*T. aestivum* or *T. spelta*), farming system (organic or conventional), and flour type (white or wholemeal) (Table 4.1). Cereal brands were used as replicates, with only one samples per brand (supermarket own or manufacturers brands) being used for each combination of wheat species, farming system, flour type per year. This was primarily done to avoid pseudo-replication, since the use of more than one sample per brand could have resulted in both flour samples originating from the same batch of grain used by the miller; different brands were assumed to have been made by different mills or at least different grain batches. Due to the small number of spelt wheat brands/samples that could be used as replicate samples found in the UK during the first year, the sample collection area for spelt wheat was extended to Germany in 2016. As a result it was possible to include an additional factor (country) in the statistical analyses of data from the 2016 and 2017 samples. As shown in table 4.1, in total, 352 samples were purchased from supermarkets in the UK (Tesco, Waitrose, Sainsbury, Marks & Spencer, Holland & Barrett) and Germany (Aldi, Biomarket, Bundnikowski, Demeter, Denn's Biomarket, Dm, Edeka, Kaufland, LIDI, Nahkauf, Netto, NP-Discount, Reformhaus, Rewe) and websites in the UK (Wessex Mill, buywholefoodonline, Shipton Mill online, Allinson, Wessex Mill, buywholefoodonline, Shipton Mill online, Gilchester online, Amazon, Matthews Gotswold. Sharpham Park, Bacheldre Watermill) in the same period in each year. Parts of samples were shown in figure 4.1. Samples were unpacked from original packages and transferred to vacuum food bags then stored in a - 80 °C freezer in containers with silica gel until analysis.

Figure 4.1 Part of flour samples collected for the project

Parameters assessed in all flour samples included (a) estimated phenolic phytochemical contents (including total phenolic content, total flavonoid and phenolics profile by HPLC), total antioxidant capacity by TEAC and FRAP and mineral concentration (including N, Na, P, K, S, Ca, Mn, Na, Cu, Fe, Zn, Mo, Ni, Al and Cd); (b) mycotoxins (DON, ZEA, OTA and T-2/HT-2) and (c) pesticide residues (Figure 4.2). It is important to note that flavonoids are one of the main classes of phenolic phytochemicals but not the only one found in wheat grains. In addition, not all phenolics in grains could be identified by HPLC in this study. However, in the thesis, for the convenience of the description and reviewing in the thesis, the definition of "estimated concentrations of phenolic phytochemicals" here includes all total phenolic content by Folin-ciocalteu method, total flavonoid content by the aluminium chloride method and total phenolic content detected by HPLC though this lacks some precision and accuracy to some extent.

Estimated concentrations of phenolic phytochemicals, total antioxidant capacity (TEAC and FRAP assays) and concentrations of minerals (including N, Na, P, K, S, Ca, Mn, Na, Cu, Fe, Zn, Mo, Ni, Al and Cd) were analysed in samples from 2015 and 2016 only (Table 4. 2). Mycotoxins were analysed in samples from all three years (Table 4.1). Pesticide assessment was carried for samples from 2016 and 2017 (Table 4. 3). Samples from 2015 were excluded from pesticide analyses, because a suitable lab for pesticide analysis was only identified in 2016, and the long storage time may have affected the pesticide residue concentrations.

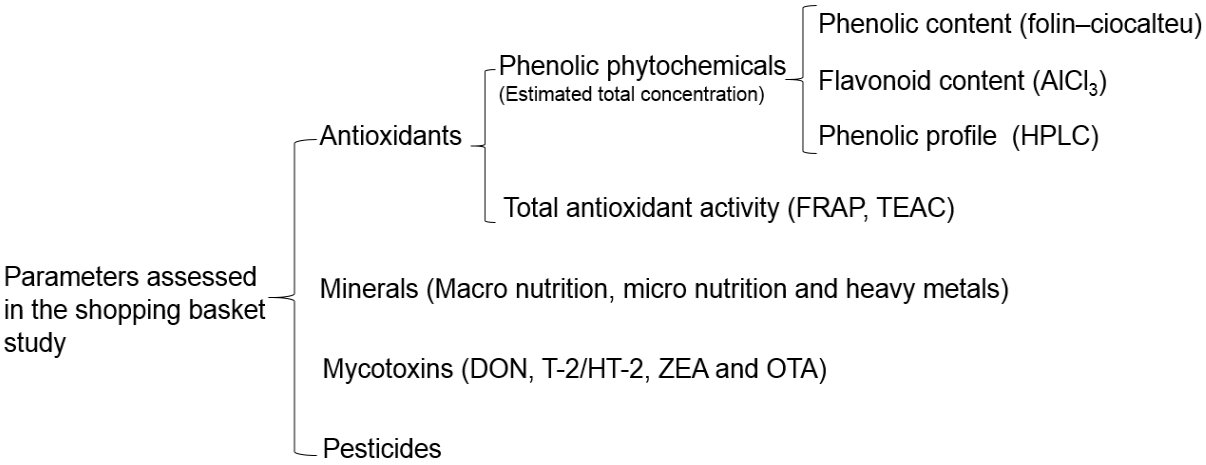


Figure 4.2 Parameters assessed in the SBS and definition of the parameters

4.4 Method

See Chapter 2 methodology

Table 4.1 Shopping basket survey samples collected in 2015, 2016 and 2017; all samples used for mycotoxin assessment.

Common wheat			**2015**	**2016**	**2017**	TOTAL
Germany	**Conventional**	**White**	0	12	28	**40**
		wholemeal	0	3	6	**9**
	Organic	**White**	0	9	20	**29**
		wholemeal	0	6	9	**15**
UK	**Conventional**	**White**	12	15	33	**60**
		wholemeal	8	11	12	**31**
	Organic	**White**	7	11	16	**34**
		wholemeal	8	10	10	**28**
		TOTAL	**35**	**77**	**134**	**246**

Spelt wheat			**2015**	**2016**	**2017**	TOTAL
Germany	**Conventional**	**White**	4	7	9	**20**
		wholemeal	2	3	5	**10**
	Organic	**White**	2	4	12	**18**
		wholemeal	2	6	9	**17**
UK	**Conventional**	**White**	1	0	2	**3**
		wholemeal	1	5	2	**8**
	Organic	**White**	2	4	4	**10**
		wholemeal	6	7	7	**20**
		TOTAL	**20**	**36**	**50**	**106**

TOTAL	***55***	***113***	***184***	***352***

Table 4.2 Shopping basket survey samples used for assessments of crude protein, phenolics, heavy metals and micronutrients

Common wheat			**2015**	**2016**	TOTAL
Germany	**Conventional**	**White**	0	12	**12**
		wholemeal	0	3	**3**
	Organic	**White**	0	9	**9**
		wholemeal	0	6	**6**
UK	**Conventional**	**White**	12	15	**27**
		wholemeal	8	11	**19**
	Organic	**White**	7	11	**18**
		wholemeal	8	10	**18**
		TOTAL	**35**	**77**	**112**

Spelt wheat			**2015**	**2016**	TOTAL
Germany	**Conventional**	**White**	4	7	**11**
		wholemeal	2	3	**5**
	Organic	**White**	2	4	**6**
		wholemeal	2	6	**8**
UK	**Conventional**	**White**	1	0	**1**
		wholemeal	1	5	**6**
	Organic	**White**	2	4	**6**
		wholemeal	6	7	**13**
		TOTAL	**20**	**36**	**56**

TOTAL	***55***	***113***	***168***

Table 4.3 Shopping basket survey samples used for pesticide assessments

Common Wheat			**2016**	**2017**	TOTAL
Germany	**conventional**	**white**	12	28	**40**
		wholemeal	3	6	**9**
	Organic	**white**	9	20	**29**
		wholemeal	6	9	**15**
UK	**conventional**	**white**	15	33	**48**
		wholemeal	11	12	**23**
	Organic	**white**	11	16	**27**
		wholemeal	10	10	**20**
		TOTAL	**77**	**134**	**211**

Spelt Wheat			**2016**	**2017**	TOTAL
Germany	**conventional**	**white**	7	9	**16**
		wholemeal	3	5	**8**
	Organic	**white**	4	12	**16**
		wholemeal	6	9	**15**
UK	**conventional**	**white**	0	2	**2**
		wholemeal	5	2	**7**
	Organic	**white**	4	4	**8**
		wholemeal	7	7	**14**
		TOTAL	**36**	**50**	**86**

TOTAL			***113***	***184***	***297***

4.5 Effect of farming system, species and flour type on phenolic phytochemical, total antioxidant capacity and mineral concentration of flours

4.5.1 Results

In the SBB, flour samples for two years taken in 2015 and 2016 were available for the analysis of phenolic phytochemical content (including total phenolic content, total flavonoid and phenolics profile by HPLC), total antioxidant capacity by TEAC and FRAP and mineral concentration (including N, Na, P, K, S, Ca, Mn, Na, Cu, Fe, Zn, Mo, Ni, Al and Cd) in all free, bound and conjugated fractions. However, no common wheat flour samples from Germany were available for analyses in 2015. It was not possible to include all experimental factors (year, country, wheat species, farming system and flour type) in the same AVOVA. Therefore, years and country were used as replicates and a 3-factor ANOVA with wheat species, farming system and flour type was carried out.

In the main thesis, only (a) total phenolic content defined as the sum of free, bound and conjugated phenolic content; (b) total flavonoid concentration defined as the sum of flavonoid in free, bound and conjugated fractions; and (c) total antioxidant capacity defined as the sum of antioxidant capacity of the free fraction, bound and conjugated fraction are presented in the main body of the thesis (Table 4.4). The results for individual free, bound and conjugated fraction are presented only in the appendices (see appendices 4.1 to 4.10).

For the phenolic profile, seven phenolics in common wheat and spelt wheat flour were detected and quantified by HPLC with a decreasing order of ferulic acid (83%) > sinapic acid (7%) > *p*-coumaric acid (4%) > 4-hydroxybenzoic acid (2%) = vanillic acid (2%) = syringic acid (2%). It could be seen that ferulic acid was the most abundant phenolic acid in common and spelt wheat, ranging from 70% to 91% of the total detected phenolic acids determined in this study. Therefore, only results for ferulic acid and total phenolics concentration detected by HPLC (the sum of ferulic acid, sinapic acid, *p*-coumaric acid, 4-hydroxybenzoic acid, vanillic acid, syringic acid and syringaldeyde) are presented and discussed in the main thesis. The results for other phenolic components are presented only in the appendices (see appendices 4.11 to 4.38)

In addition, for convenience of the description and reviewing in the thesis, “phenolic phytochemicals” was used to indicate all the assessed parameters including total phenolic content, total flavonoid content, ferulic acid concentration, total phenolics concentration detected by HPLC. Furthermore, it needs to be noted that the terms, “total phenolic content” and “total phenolic compounds” in the following sections are used to distinguish the total phenolic content (TPC) assessed by using the Folin-Ciocalteu colorimetric assay and the sum of all phenolics concentration detected by and HPLC, respectively.

Phenolic phytochemicals and Antioxidant capacity

Significant main effects of farming system and flour type were detected for all detected phenolic phytochemicals (including total phenolic content, total flavonoid, **ferulic acid**, and total phenolic compounds detected by HPLC) and total antioxidant capacity (FRAP and TEAC), with higher phenolic phytochemical concentration and antioxidant capacity found to be between 10 and 33% higher in organic compared with conventional flour and between 2 and 5 times higher in wholegrain compared with white flour (Table 4.4). Significant main effects of wheat species were only detected for total phenolic contents by colorimetric assay and antioxidant capacity (TEAC), with spelt having 11% higher phenolic and 15% higher antioxidant capacity (TEAC) (Table 4.4).

A significant 2-way interaction between farming system and flour type was only detected for antioxidant capacity (FRAP) (Table 4.4). Antioxidant capacity was significantly higher in organic than conventional wholemeal but not white flour (Table 4.8).

Significant 2-way interactions between wheat species and flour type were detected for concentrations of all phenolic phytochemicals (except flavonoids) and antioxidant capacity (FRAP and TEAC) (Table 4.4). When wholegrain flours were compared, common wheat flour had significantly higher phenolic phytochemical concentrations and antioxidant capacity than spelt flour. In contrast, when white flours were compared, spelt flour had numerically higher phytochemical concentrations and antioxidant capacity, but the difference was only significant for total phenolic content (Table 4.9).

Significant 3-way interactions were detected for total flavonoids, ferulic acid concentrations and total phenolic compounds detected by HPLC (Table 4.4). Significantly higher flavonoid concentration in organic compared with conventional samples were only detected for white common wheat flour. In contrast, significantly higher ferulic acid concentrations in conventional compared with organic samples were only detected for wholegrain common wheat flour. For all other flour types there were no significant differences between organic and conventional samples (Table 4.10).

When concentrations of free, bound and conjugated phenolics and flavonoid, and antioxidant capacity in these fractions were compared, overall trends were similar to those found for total concentrations and activity (Appendices 4.1-4.38).

Pearson correlation analysis was performed to correlate the results obtained with the different analytical methods (Table 4.11a). Between **total antioxidant capacity by FRAP and TEAC:** Antioxidant capacity obtained by FRAP and TEAC methods were significantly and very strongly positively correlated ($R=0.859$, $P<0.01$). Between **TPC and Antioxidant capacity**: A strong positive correlation was found between the total antioxidant capacity and TPC

(R=0.883, P>0.05 for TPC vs FRAP, and R=0.770, P>0.05 for PC vs TEAC). **PC, AA and Flavonoid:** For flavonoid, relatively lower but still positive correlations were found for total antioxidant capacity and TPC with the lowest correlation coefficient R= 0.504 (P<0.01) between TPC and flavonoid and the highest R=0.621 (P<0.01) between FRAP and flavonoid (Table 4.11a). Similar overall correlations between results of these different analytical methods were found when Pearson correlation was performed for free, bound and conjugated fractions separately (Appendix 4.39).

Crude protein and mineral macro-nutrient concentrations

Crude protein concentrations were estimated based on nitrogen (N) concentrations in grains and ANOVA results were therefore identical for crude protein and N-content (Table 4.5). Significant main effects were detected for all 3 factors, and crude protein/N concentrations were significantly higher in spelt, conventional and wholemeal flour than in common wheat, organic and white flour (Table 4.5). A significant 2-way interaction between wheat species and flour type was also detected (Table 4.5), with significantly higher crude protein/N concentrations in wholegrain flour than in white flour being detected in common wheat, but not spelt wheat samples (Table 4.9).

Sodium (Na): Significant main effects of wheat species and flour type were detected for Na concentrations, which were significantly higher in wheat flours than in spelt flours (46%) and in wholemeal flours compared with white flours (9%) (Table 4.5). There was a 2-way interaction between wheat species and flour type with significantly higher sodium concentrations in common wheat than in spelt wheat only in wholemeal flour but not in white flour (Table 4.9).

Phosphorus (P), potassium (K), Magnesium (Mg) Sulphur (S): Significant main effects of all 3 experimental factors were detected for P, K and Mg concentrations, which were significantly higher in spelt flour compared with common wheat flour (38%, 27%, and 42% respectively), in organic flour compared with conventional flour (41%, 27%, and 49% respectively) and in wholemeal flour compared with white flour (148%, 125%, and 209% respectively). Similar trends were also detected for S, but main effects were only significant for wheat species and flour type; S concentrations were also significantly higher in spelt (29%) compared with common wheat flours and in wholemeal flour (10%) compared with refined flour (Table 4.5).

For K and Mg, there were significant 2-way interactions between farming system and flour type, with significantly higher K and Mg concentrations in organic compared with conventional flour being detected in wholegrain, but not white flour samples (Table 4.8).

For S there were significant 2-way interactions between (a) wheat species and farming system and (b) wheat species and flour type. Significantly higher S-concentrations were detected in

organic spelt flour, but conventional common wheat flour (Table 4.7). Also, higher S-concentrations were found in white spelt flour compared with wholemeal spelt, and in wholegrain common wheat flour compared with white common wheat flour, but the difference was only significant for common wheat (Table 4.9).

Calcium (Ca): The trends for Ca were different to those observed for all other mineral macronutrients. Significant main effects of wheat species and flour type were detected, with concentrations found to be higher in common wheat and white flour (97% and 69% respectively) compared with spelt wheat flours and wholemeal flours (Table 4.5).

Mineral micro-nutrients

Significant main effects of all 3 experimental factors were detected for manganese (Mn), zinc (Zn), copper (Cu) and Molybdenum (Mo) concentrations, which were significantly higher in spelt flour compared with common wheat flour (31%, 64%, 35% and 24% respectively), in organic flour compared with conventional flour (41%, 27%, and 49% respectively) and in wholemeal flour compared with white flour (148%, 125%, and 209% respectively). Similar trends were also detected for Iron (Fe), but main effects were only significant for wheat species and flour type; Fe concentrations were also significantly higher in organic flour compared with conventional flour (16%) and in wholemeal flour compared with white flour (63%) (Table 4.6).

For Cu a significant 2-way interaction between wheat species and farming systems was detected (Table 4.6), with concentrations significantly higher in organic common wheat flour than in conventional common wheat, but not different in spelt wheat flour (Table 4.8). For Mo a significant 2-way interaction between wheat species and flour type was detected (Table 4.6), with concentrations significantly higher in spelt white flour than in common white wheat flour, but not wholegrain flours (Table 4.9). For Mn, Zn and Cu significant 2-way interactions were detected between farming systems and flour type (Table 4.6), with concentrations significantly higher in organic than conventional wholegrain flour, but not different in white flour (Table 4.8).

Undesirable and toxic metals

A significant main effect of wheat species was only detected for the toxic metal cadmium (Cd), with concentrations found to be significantly higher (28%) in spelt than common wheat flour (Table 4.6). Significant main effects of farming system were detected for the undesirable metals Al and Ni, with concentrations found to be significantly higher (12% and 81% respectively) in organic than in conventional flour (Table 4.6).

For Al there were significant 2-way interactions between (a) wheat species and farming system and (b) farming system and flour type. Al concentration were found to be significantly higher in organic common wheat flour than in conventional common wheat, but no differences

between spelt wheat flours. Also, common wheat had higher Al concentrations then spelt wheat flour when organic, but not when conventional samples were compared (Table 4.7). Al concentrations were found to be significantly higher in organic than conventional wholegrain but not white four samples (Table 4.8).

Table 4.4 Main effect means ± SE and *p*-values for the effects and interaction between wheat species, farming system and flour type on phytochemical concentrations and total antioxidant capacity (FRAP, TEAC)

	Colorimetric Assays		**HPLC**		**Colorimetric Assays**	
	total phenolic content	**flavonoids**	**ferulic acid**	**total phenolic compounds***	**Total antioxidant capacity**	
					FRAP	**TEAC**
Factor	µmol GAE g^{-1} flour (DW)	µmol catechin g^{-1} flour (DW)	µmol g^{-1} flour (DW)		µmol $FeSO_4\,7H_2O$ g^{-1} flour (DW)	µmol Trolox g^{-1} flour (DW)
Farming system						
Conventional (n=84)	8.4 ±0.4	0.83 ±0.08	334 ±34	394.43±39.55	4.3 ±0.36	7.8 ±0.6
Organic (n=83)	9.2 ±0.4	1.10 ±0.09	382 ±31	456.95±35.73	5.5 ±0.40	10.1 ±0.7
Species						
Spelt (n=55)	9.4 ±0.4	0.98 ±0.08	369 ±34	438.43±39.07	5.1 ±0.41	9.8 ±0.8
Wheat (n=112)	8.5 ±0.4	0.95 ±0.08	352 ±30	419.15±34.98	4.8 ±0.35	8.5 ±0.6
Flour Type						
White (n=90)	6.0 ±0.2	0.57 ±0.07	120 ± 8	148.65±10.44	2.0 ±0.13	3.9 ±0.2
Wholemeal (n=77)	12.1 ±0.3	1.42 ±0.07	636 ±23	749.10±25.90	8.3 ±0.22	14.8 ±0.4
ANOVA- results (p-values)						
Main Effects						
Farming System (PS)	**0.0232**	**0.0094**	**0.0887**	***0.0502***	**<0.0001**	**<0.0001**
Species (SP)	**0.0053**	NS	NS	NS	NS	**0.038**
Flour Type (FT)	**<0.0001**	**<0.0001**	**<0.0001**	**<.0001**	**<0.0001**	**<0.0001**
Interactions						
FS × SP	NS	NS	NS	NS	NS	NS
FS × FT	*0.0742*	NS	NS	NS	**0.0131** [1]	*0.075*
SP × FT	**<0.0001** [2]	NS	**0.0002** [2]	**0.0001**	**0.0002** [2]	**0.0112** [2]
FS × SP × FT	NS	**0.0198** [3]	**0.0062** [3]	**0.0109** [3]	NS	NS

[1], see Table 4.8 for interaction means ± SE; **[2]**, see Table 4.9 for interaction means ± SE; **[3]**, see Table 4.10 for interaction means ± SE;
Total phenolic compounds by HPLC is the sum concentration of protocatechuic acid, 4-hydroxybenzoic acid, vanillic acid, syringic acid, p-coumaric acid, syringaldehyde , sinapic acid and ferulic acid detected by HPLC.

Table 4.5 Main effect means ± SE and *p*-values for the effects and interaction between year, wheat species, farming system and flour type on macro nutrition in flour collected from UK and DE between 2015 and 2016

	Crude	**mineral macro-nutrients**						
	Protein	**N**	**Na**	**P**	**K**	**S**	**Ca**	**Mg**
Factor	**%**	mg/g	mg/kg	mg/g	mg/g	mg/g	mg/g	mg/g
Farming system								
Conventional (n=82)	11.3 ±0.19	17.9 ±0.30	40.2 ±2.9	1.3 ±0.09	1.3 ±0.08	0.82 ±0.03	0.48 ±0.05	0.35 ±0.03
Organic (n=80)	10.5 ±0.17	16.7 ±0.27	36.7 ±2.6	1.8 ±0.12	1.7 ±0.10	0.86 ±0.03	0.48 ±0.05	0.52 ±0.04
Species								
Spelt (n=54)	11.5 ±0.17	18.2 ±0.27	29.5 ±1.4	1.9 ±0.13	1.8 ±0.11	0.99 ±0.04	0.29 ±0.03	0.54 ±0.04
Wheat (n=108)	10.7 ±0.17	16.9 ±0.27	43.1 ±2.8	1.3 ±0.09	1.4 ±0.08	0.77 ±0.02	0.57 ±0.05	0.38 ±0.03
Flour Type								
White (n=87)	10.5 ±0.18	16.6 ±0.28	37.0 ±2.4	0.9 ±0.05	1.0 ±0.04	0.80 ±0.03	0.59 ±0.05	0.22 ±0.01
Wholemeal (n=75)	11.5 ±0.18	18.2 ±0.28	40.2 ±3.2	2.2 ±0.11	2.2 ±0.08	0.88 ±0.03	0.35 ±0.05	0.68 ±0.03
ANOVA- results (p-values)								
Main Effects								
Farming System (FS)	**0.0001**	**0.0001**	NS	**<.0001**	**0.0001**	NS	NS	**<.0001**
Species (SP)	**0.0003**	**0.0003**	**0.0023**	**<.0001**	**0.0004**	**<.0001**	**0.0013**	**<.0001**
Flour Type (FT)	**<.0001**	**<.0001**	**0.0228**	**<.0001**	**<.0001**	**<.0001**	**0.0022**	**<.0001**
Interactions								
FS × SP	NS	NS	NS	NS	NS	**0.0014** [1]	NS	NS
FS × FT	NS	NS	NS	NS	**0.0339** [2]	NS	NS	**0.0155** [2]
SP × FT	**0.0656** [3]	**0.0656** [3]	**0.0366** [3]	NS	NS	**0.0036** [3]	NS	NS
FS × SP × FT	NS	NS	NS	*0.0853*	NS	*0.0891*	NS	NS

[1], see Table 4.7 for interaction means ± SE; [2], see Table 4.8 for interaction means ± SE; [3], see Table 4.9 for interaction means ± SE;

Table 4.6 Main effect means ± SE and *p*-values for the effects and interaction between wheat species, farming system and flour type on micronutrients and undesirable/toxic metals in flour collected in the UK and DE in 2015 and 2016

	mineral micronutrients					**undesirable and toxic metals**		
	Fe	**Mn**	**Zn**	**Cu**	**Mo**	**Al**	**Ni**	**Cd**
	mg/kg	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**mg/kg**	**µg/kg**
Farming system								
Conventional (n=82)	19 ±1.0	9.9 ±0.8	11 ±0.7	3.7 ±0.2	0.27±0.01	3.7 ±0.2	0.26±0.02	38 ±2
Organic (n=80)	22 ±1.2	14.9 ±1.1	16 ±1.0	5.3 ±0.3	0.46±0.03	4.6 ±0.3	0.47±0.08	42 ±2
Species								
Spelt (n=54)	22 ±1.4	14.7 ±1.3	18 ±1.2	5.4 ±0.4	0.41±0.03	3.8 ±0.3	0.46±0.05	46 ±3
Wheat (n=108)	20 ±1.0	11.2 ±0.9	11 ±0.6	4.0 ±0.2	0.33±0.02	4.3 ±0.3	0.32±0.06	36 ±2
Flour Type								
White (n=87)	16 ±0.8	6.2 ±0.4	9±0.5	3.7 ±0.2	0.30±0.02	4.2 ±0.23	0.36±0.07	38 ±2
Wholemeal (n=75)	26 ±1.2	19.6 ±1.0	19±0.9	5.5 ±0.3	0.43±0.03	4.1 ±0.32	0.36±0.04	42 ±2
ANOVA- results (p-values)								
Main Effects								
Farming System (FS)	**0.0123**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**0.0094**	**0.0216**	NS
Species (SP)	NS	**0.0015**	**<.0001**	**0.0024**	**0.0096**	NS	NS	**0.0041**
Flour Type (FT)	**<.0001**	**<.0001**	**<.0001**	**<.0001**	**<.0001**	NS	NS	*0.0548*
Interactions								
FS × SP	NS	NS	NS	**0.0242** [1]	NS	**0.0109** [1]	NS	NS
FS × FT	NS	**0.0024** [2]	**0.0006** [2]	**0.0324** [2]	NS	**0.0015** [2]	NS	NS
SP × FT	NS	NS	NS	NS	**0.0465** [3]	NS	NS	NS
FS × SP × FT	NS	NS	NS	*0.0768*	NS	NS	NS	NS

[1], see Table 4.7 for interaction means ± SE; **[2]**, see Table 4.8 for interaction means ± SE; **[3]**, see Table 4.9 for interaction means ± SE;

Table 4.7 Interactions means ± SE for the effects of species and flour type on sulphur, copper and aluminium concentrations in flour collected from UK and DE in 2015 and 2016.

	Factor 1	**Factor 2 farming system**	
Parameters assessed	**wheat species**	organic	conventional
Sulphur	spelt	0.94 ±0.05 **B a**	1.05 ±0.05 **A a**
mg g^{-1}	common	0.80 ±0.03 **A b**	0.74 ±0.03 **B b**
Copper	spelt	5.8 ±0.6 **A a**	5.2 ±0.5 **A a**
mg kg^{-1}	common	5.0 ±0.3 **A a**	3.2 ±0.2 **B b**
Aluminium	spelt	3.5 ±0.4 **A b**	4.1±0.4 **A a**
mg kg^{-1}	common	5.2 ±0.4 **A a**	3.6±0.3 **B a**

For each parameter assessed means labelled with the same capital letter within rows and lower case letter within columns are not significant different (Tukey's honestly significant difference test $P<0.05$)

Table 4.8 Interactions means ± SE for the effects of flour type and farming system on antioxidant capacity and mineral content in flour collected from UK and DE in 2015 and 2016.

	Factor 1	**Factor 2 farming system**	
Parameters assessed	**flour type**	organic	conventional
Antioxidant capacity FRAP µmol $FeSO_4\ 7H_2O\ g^{-1}$ flour (DW)	white	2.0 ±0.2 **A b**	2.0 ±0.2 **A b**
	wholegrain	8.7 ±0.3 **A a**	7.8 ±0.3 **B a**
Potassium mg g^{-1}	white	1.0 ±0.05 **A b**	0.9 ±0.07 **A b**
	wholegrain	2.3 ±0.11 **A a**	2.0 ±0.12 **B a**
Magnesium mg g^{-1}	white	0.24 ±0.02 **A b**	0.21 ±0.02 **A b**
	wholegrain	0.76 ±0.04 **A a**	0.58 ±0.04 **B a**
Manganese mg kg^{-1}	white	7 ±0.6 **A b**	6 ±0.6 **A b**
	wholegrain	22 ±1.3 **A a**	16 ±1.2 **B a**
Zinc mg kg^{-1}	white	9 ±0.7 **A b**	8 ±0.6 **A b**
	wholegrain	22 ±1.0 **A a**	15 ±1.1 **B a**
Copper mg kg^{-1}	white	4.1 ±0.3 **A b**	3.3 ±0.3 **A b**
	wholegrain	6.3 ±0.4 **A a**	4.4 ±0.3 **B a**
Aluminium mg kg^{-1}	white	4.2 ±0.4 **A a**	4.2 ±0.3 **A a**
	wholegrain	4.9 ±0.5 **A a**	3.0 ±0.3 **B a**

For each parameter assessed means labelled with the same capital letter within rows and lower case letter within columns are not significant different (Tukey's honestly significant difference test $P<0.05$)

Table 4. 9 Interactions means ± SE for the effects of species and flour type phenolic phytochemical contents, antioxidant capacity (FRAP and TEAC) and mineral contents in flour collected from UK and DE in 2015 and 2016.

	Factor 1	**Factor 2 flour type**	
Parameters assessed	**wheat species**	white	wholegrain
Total phenolic content (Colorimetric)	spelt	7.1 ±0.4 **B a**	11.2 ±0.4 **A b**
µmol GAE g^{-1} flour (DW)	common	5.6 ±0.2 **B b**	12.7 ±0.4 **A a**
Total ferulic acid (HPLC)	spelt	131 ±24 **B a**	554 ±26 **A b**
µmol g^{-1} flour (DW)	common	116 ± 7 **B a**	691 ±32 **A a**
Total sinapic acid (HPLC)	spelt	14 ±3.3 **B a**	39 ±2.4 **A b**
µmol g^{-1} flour (DW)	common	11 ±1.1 **B a**	54 ±2.5 **A a**
Antioxidant capacity FRAP	spelt	2.2 ±0.30 **B a**	7.4±0.28 **A b**
µmol $FeSO_4\,7H_2O$ g^{-1} flour (DW)	common	1.9 ±0.14 **B a**	8.9±0.28 **A a**
Antioxidant capacity TEAC	spelt	4.5 ±0.5 **B a**	13.8 ±0.6 **A b**
µmol Trolox g^{-1} flour (DW)	common	3.7 ±0.2 **B a**	15.4 ±0.6 **A a**
Nitrogen	spelt	17.8 ±0.4 **A a**	18.4 ±0.4 **A a**
mg g^{-1}	common	16.1 ±0.3 **B b**	17.9 ±0.4 **A b**
Sulphur	spelt	1.04 ±0.05 **A a**	0.95 ±0.05 **A a**
mg g^{-1}	common	0.72 ±0.03 **B b**	0.84 ±0.04 **A a**
Molybdenum	spelt	0.38 ±0.03 **A a**	0.44 ±0.05 **A a**
mg kg^{-1}	common	0.27 ±0.02 **B b**	0.43 ±0.03 **A a**

For each parameter assessed means labelled with the same capital letter within rows and lower case letter within columns are not significant different (Turkey's honestly significant difference test $P<0.05$)

Table 4.10 Interactions means ± SE for the effects of species, farming system and flour type on the total flavonoid, ferulic content and total concentration of phenolic acids detected by HPLC of flour collected from UK and DE between 2015 and 2016

Parameter assessed	**Factor 1 species**	**Factor 2 flour type**	**Factor 3 farming system** organic	conventional
		Colorimetric Assays		
Total	spelt	White	0.46 ±0.07 **A b**	0.60 ±0.09 **A b**
flavonoids		Wholegrain	1.49 ±0.11 **A a**	1.05 ±0.11 **A a**
µmol catechin	wheat	White	0.78 ±0.20 **A b**	0.45 ±0.05 **B b**
g^{-1} flour (DW)		Wholegrain	1.45 ±0.08 **A a**	1.51 ±0.21 **A a**
		HPLC		
Total	spelt	White	102 ±16 **A b**	159 ±45 **A c**
Ferulic acid		Wholegrain	579 ±25 **A a**	509 ±58 **A b**
µmol	wheat	White	123 ±12 **A b**	112 ± 9 **A c**
g^{-1} flour (DW)		Wholegrain	650 ±36 **B a**	735 ±52 **A a**
Total phenolic	spelt	White	132 ±20 **A c**	197 ±56 **A c**
compounds		Wholegrain	677 ±29 **A b**	603 ±66 **A b**
µmol	wheat	White	153 ±15 **A c**	136 ±12 **A c**
g^{-1} flour (DW)		Wholegrain	779 ±41 **A a**	856 ±58 **A a**

For each parameter assessed means labelled with the same capital letter within rows and lower case letter within columns are not significant different (Turkey's honestly significant difference test P<0.05)

Table 4.11a Correlation coefficients between total phenolic content (TPC) and antioxidant capacity and between different measure of antioxidant capacity

TPC vs FRAP	TPC vs TEAC	TPC vs FLA	FRAP vs TEAC	FRAP vs FLA	TEAC vs FLA
0.883**	0.700**	0.504**	0.859**	0.621**	0.543**

*,** were significant at 0.05, 0.01 probability level, respectively

4.5.2 Discussion

Overall, results from this study suggest that there are significant differences in phenolic phytochemicals and mineral composition between and (a) organic and conventional flour (b) spelt and common wheat, and that refining of grains (removal of most of the bran and germ) during the production of white flour removes a large proportion of these nutrients (Tables 4.4, 4.5 and 4.6) (Kim *et al.*, 2006; Vaher *et al.*, 2010; Cho *et al.*, 2013; Eagling *et al.*, 2014; Oghbaei and Prakash, 2016; Vignola *et al.*, 2016; Ertl and Goessler, 2018). The relative impacts and interactions between agronomic practices ,wheat genetics and grain processing on the nutritional quality and associated potential health impacts is discussed in separate sections below.

Effect of farming systems (organic versus conventional)

The finding of higher phenolic phytochemical, Mg and Zn concentration, and/or antioxidant capacity in organic compared with conventional wheat (Tables 4.4, 4.5 and 4.6), is consistent with the results of a meta-analysis of data from 343 peer-reviewed publications, which reported that phenolic phytochemical concentrations are higher in organic than in conventional crops (Baranski *et al.*, 2014). However, the meta-analysis and a recent factorial field experiment (Cooper *et al.*, 2013) also suggested that concentrations of the toxic metal cadmium are significantly lower in organic cereals crops, while both organic and conventional flours had similar Cd concentrations in the SBB reported here (Table 4.6). Also, concentrations of the nutritionally undesirable elements Al and Ni were significantly higher in organic wheat flour (Table 4.6). This represents some negative trade-off for the higher antioxidant levels in organic flour, but is unlikely to have a nutritional/health impact given the relatively low concentrations of Al and Ni found in flour and the level of difference observed (Trumbo *et al.*, 2001; Hardisson *et al.*, 2017).

There is evidence that use of mineral N-fertiliser, herbicides and modern short-straw varieties can all have a negative effect on phenolic phytochemical concentrations in wheat. Mineral N-fertiliser use was reported as a major driver for the lower concentrations of phenolic phytochemicals found in both grains and leaves of conventional cereal crops (Almuayrifi, 2013; Baranski *et al.*, 2014; Rempelos *et al.*, 2018a). Also, higher concentrations of phenolic acids and flavonoids in leaves of organic crops were linked to higher levels of resistance against biothrophic cereal diseases such as mildew and rust (Nicholson and Hammerschmidt, 1992; Bennett and Wallsgrove, 1994; Sander and Heitefuss, 1998; Stewart *et al.*, 2001; Almuayrifi, 2013; Baranski *et al.*, 2014). Daniel *et al.* (1999) reported that herbicides reduce phenolic compounds and other secondary metabolites levels in plants. A recent study also reported that moderns short-straw, UK common wheat varieties have lower phenolic acid and flavonoid

concentrations than longer-straw varieties developed recently for organic farming systems, and that the relative difference is substantially greater when composted manure rather and mineral N is used as fertiliser (Almuayrifi, 2013). Longer straw US common wheat varieties developed in the 1960s were also shown to have higher mineral micronutrient concentrations than modern short straw varieties currently used in the US (Murphy *et al.*, 2008).

However, since detailed information on the agronomic practices and wheat varieties used to make the flour assessed in this study was not available, the relative effect of genetic and different agronomic drivers on wheat flour composition cannot not be determined

Effect of wheat species (common vs spelt wheat)

Results from this study suggest that spelt flour had significantly higher phenolic phytochemical concentrations and antioxidant capacity than common wheat, and that the differences were greater in wholemeal than in white flour (Table 4.4). Concentrations of protein/N, S, and all mineral micro-nutrients were significantly higher in spelt than common wheat (Tables 4.5 and 4.6). The results reported here are not consistent with previous studies that compared the composition of spelt and common wheat flour. For example, two previous studies reported no significant differences in phenolic phytochemical levels between species (Abdel-Aal and Rabalski, 2008; Li *et al.*, 2008), while Calzuola *et al.* (2013) reported significantly higher flavonoid concentrations in spelt wheat than in common wheat, but no significant differences in total phenolic content.

All previous comparative studies used milled wholegrain or wholegrain wheat products made from a range of different *T. aestivum* and *T. spelta* and it is likely that they were affected by within species variation, which can be considerable (Abdel-Aal and Rabalski, 2008; Li *et al.*, 2008; Hussain *et al.*, 2012; Calzuola *et al.*, 2013). Another limitation of most previous studies that may explain differences in outcomes is that they only assessed or reported concentrations of free phenolic acids (Van Hung, 2014), while in the study reported here, free, bound and conjugated phenolics, and flavonoids were quantified and were found to be different.

Effect of grain refining/processing (white vs wholegrain flour)

The results of this study which showed that wholemeal flour has a higher nutritional value than white flour (tables 4.4, 4.5 and 4.6) is consistent with previous studies, which reported that wholemeal flours and cereal products made from them have a substantially higher protein, fibre, phenolic phytochemical and mineral macro-, and micronutrient content than white (refined) flour and cereal products (Vaher *et al.*, 2010; Borneo and Len, 2012; van der Kamp *et al.*, 2014; Oghbaei and Prakash, 2016). This study also suggests that refining (removal of the bran and germ) has (a) a substantially greater impact on the mineral nutrients and phenolic phytochemical concentrations in flour than wheat genetics (*T. aestivum* vs *T. spelta*) or

production protocols (organic vs conventional), but does not (b) increase concentrations of nutritionally undesirable and/or toxic metals (Al, Ni, Cd) in flour (Kirleis *et al.*, 1984; Albergamo *et al.*, 2018; Ertl and Goessler, 2018). The finding of similar concentrations of Al, Ni and Cd in white and wholegrain flour suggests that these nutritionally undesirable compounds may be more evenly distributed between the endosperm, germ and bran fraction of the grain, than other mineral macro and micro nutrients, but this needs further investigation (Albergamo *et al.*, 2018).

The finding that phenolic phytochemicals (not including flavonoids) and antioxidant capacity (FRAP and TEAC) were higher in common wholegrain wheat flour than in wholegrain spelt flour, but were higher in white spelt than in common wheat white flour is reported for the first time here (Table 4.4) (Zieliński *et al.*, 2008b). It suggests that the negative impact of grain refining on phenolic phytochemical levels is greater in common wheat than in spelt wheat. This view is supported by the finding that the difference in protein/N concentrations between wholemeal and white flour was also greater in common (11%) than in spelt (3%) wheat. This could be due to differences in (a) grain physiology/morphology and/or (b) the refining process (for spelt an additional processing step is required to remove the husk; (Longin *et al.*, 2016; Baker, 2018) used for the two species, that result in different amounts of bran and germ being removed in common and spelt wheat.

It is well known that changes to the milling process affect the percentage of bran and germ loss and thus when determining nutritional quality it has been recommended that studies focused on comparing the nutritional composition of white flour use standardised extraction and analytical methods (Shewry and Hey, 2015) to allow for more accurate comparisons between wheat species/varieties and/or farming systems.

Potential impacts on human health

Phenolics and human health: The most recent review of the role of phenolic phytochemicals in modern nutrition by Williamson (2017) describes the absorption and metabolism of phenolic phytochemicals in the body and summarizes the biological effects of phenolic phytochemicals -rich tea, coffee and cocoa indicated by human intervention studies. The gut microbiota plays a critical role in absorption of many phenolic phytochemicals and it is suggested that more than 80% of a dose can be absorbed and ultimately excreted in the urine (Williamson, 2017).

There is now substantial epidemiological evidence suggesting that a diet high in phenolic phytochemicals -rich food may protect against developing cardiovascular disease and type 2 diabetes (de Munter *et al.*, 2007; Arab *et al.*, 2009; Yang *et al.*, 2014; Jumar and Schmieder, 2016; Lee *et al.*, 2016; Martin *et al.*, 2016). However, since many of the antioxidant rich foods contain a great diversity of biologically active phytochemicals it is often difficult or impossible

to separate out the effects of individual compounds (Williamson, 2017). Also, despite extensive research, the exact mechanisms of action of phenolic phytochemicals in the human body is not completely understood, but there is strong evidence that some targets such as nitric metabolism, carbohydrate digestion and oxidative enzymes are important for the health benefits observed. It is unlikely that metabolites of polyphenolics which appear in the bloodstream retain 'antioxidant' properties, but rather the molecules may act directly as cell-signalling molecules with direct effects on cellular metabolism (Williamson, 2017).

4.5.3 Conclusion

The finding of substantially higher phenolic phytochemicals and essential mineral micronutrients such as Mg, Fe, Zn, Cu in wholegrain cereal products lends further support to current dietary recommendations to switch to wholegrain cereal product consumption.

The flour SBB reported here was based on all Spelt and Common wheat flour brands that could be found on the shelves of major UK and German retailers and can therefore be assumed to relatively accurately reflect the main wheat varieties and flour products of the flour mills/supply chains in these countries. The study also suggests that organic and spelt flour consumption would result in higher phenolic phytochemicals and mineral micronutrient intakes with associated potential health benefits.

Organic wholemeal flour allows higher intakes of high phenolic phytochemicals and mineral micro-nutrients and associated potential health benefits to be achieved without simultaneously increasing dietary exposure to pesticides; in conventional flour substantially higher pesticide residues are found in wholemeal than white flour (Wang 2018; Wang et al. 2019).

4.6 Effect of harvest year, species, farming system and flour type on mycotoxin content of common and spelt wheat flour – shopping basket survey in the UK and Germany

In the mycotoxin assessment, (a) the mean concentration of mycotoxins in flour samples, and (b) the percentage of samples testing positive for each mycotoxin contamination, were analysed.

Results from samples taken in 3 years (2015, 2016 and 2017) were available, but no common wheat flour samples from Germany were available for analyses in 2015. Consequently it was not possible to include all experimental factors (year, country, and species, farming system and flour type) in the same AVOVA. For data on the percentage of samples testing positive for mycotoxin contamination in different years we therefore used years as replicates and carried out two separate 3-factor ANOVAs with (a) wheat species, farming system and flour type (ANOVA 1) or (b) country, wheat species and farming system (ANOVA 2 as factors) (see results presented in Table 4.11). For data on the mean concentration of mycotoxins in flour samples, we used year as a random factors and carried out a 4-factor ANOVA with carried out with country, wheat species, farming system and flour type as factors (see results presented in Table 4.12).

To test for potential confounding effect of year we carried out two additional 4-factor ANOVAs with year, country, farming system and flour type as factors for common wheat and spelt wheat separately (Appendices 4.40 and 4.41). For common wheat we used data from 2016 and 2017 only, since no common wheat samples were collected in Germany in 2015 (Appendix 4.40). For spelt wheat we included data from 2015 and 2017 only since no white flour spelt wheat samples could be collected in Germany in 2016 (Appendix 4.41).

ANOVA results obtained for different mycotoxins are described in separate subsections below.

4.6.1 Results

Proportion of flour samples testing positive for mycotoxins

When the proportions of samples testing positive for different mycotoxins were compared, nearly all (99%) of samples tested positive for at least one mycotoxin (OTA) (Table 4.11). There were no significant main effects of species, farming system, flour types and country, for the prevalence of DON, ZEA and OTA. However, for T-2/HT-2 toxins a significant main effect of flour type was detected with a higher proportion of positive samples found in wholegrain flour (70%) than in white flour (51%) (Table 4.11).

There were also significant interactions between (a) farming systems and country for DON and (b) between farming system and wheat species for both T-2/HT-2 toxins and OTA (Table 4.11). DON was detected in a significantly higher number of conventional (73%) than organic

(44%) flour samples from the UK, but not for samples from Germany (41% for conventional VS 42% for organic) (Table 4.11.2). T-2/HT-2 was detected in a significantly higher proportion of organic (72%) than conventional spelt (55%), but in a significantly lower number of organic (52%) than conventional (64%) common wheat samples (Table 4.11.1). OTA was detected in a lower proportion of organic (97%) than conventional spelt (100%), but in a higher proportion of organic (100%) than conventional (98%) common wheat samples (Table 4.11.1).

Concentrations of mycotoxins in wheat flour

When the mean concentrations of *Fusarium* mycotoxins were compared, they were found to be more than 10 times lower than the MCLs set by the EU for DON and ZEA or recommended by the EU for T-2/HT-2 (Table 4.12). In contrast, OTA (a mycotoxin produced by the common moulds *Penicillium* and *Aspergillus* spp.) concentrations detected in a substantial number of flour samples exceeded the MCL (3 µg/kg) set by the EU (Table 4.12).

Significant main effects of wheat farming system, species and flour type were only detected for DON, ZEA and T-2/HT-2 toxin respectively, with conventional flour (60 µg/kg) having higher DON than organic flour (49 µg/kg), common wheat flour (3.8 µg/kg) having higher ZEA than spelt wheat flour (3.5 µg/kg) and wholegrain flour (3.8 µg/kg) having higher T-2/HT-2 concentrations than white flour (1.7 µg/kg) (Table 4.12). Significant main effects of country were detected for DON, ZEA and OTA; samples from Germany had approximately 30% lower DON and 20% lower OTA concentrations than these from UK, while samples from the UK had approximately 10% lower ZEA concentrations than those from Germany (Table 4.12).

A wide range of interactions involving all 4 experimental factors was also detected (Tables 4.12).

When the 4-way interactions detected for the common mould mycotoxin OTA and the *Fusarium* mycotoxin DON were investigated further, different trends were detected for OTA and DON (Table 4.12.1).

For DON the only flour types for which significant differences between countries could be detected were (a) conventional, wholegrain, common wheat flour (higher concentration in samples from the UK with 116 µg/kg than in samples from Germany with 28 µg/kg) and (b) organic, wholegrain, common wheat flour (higher concentrations in samples from Germany with 110 µg/kg than in the UK samples with 46 µg/kg) (Table 4.12.1). Also, DON concentrations in wholegrain, common wheat from Germany were significantly higher in organic (110 µg/kg) compared with conventional samples (28 µg/kg), while in the UK samples concentrations were significantly higher in conventional (116 µg/kg) than organic samples (46 µg/kg). For all other flour types no significant difference in DON concentrations could be

detected between farming systems, and mean concentrations for all flour types were approximately 7 times lower than the MCL of 750 µg/kg set for DON by the EU (Table 4.12.1).

For OTA all types of common wheat flour (organic and conventional, white and wholegrain) from the UK had significantly higher OTA concentrations than the same flour types collected in Germany. Also, mean OTA concentration of all types of common wheat flour collected in the UK (but not those collected in Germany exceeded the MCL for OTA of 3 µg/kg set by the EU (Table 4.12.1). However, when spelt flour samples from Germany and the UK were compared, significantly higher OTA levels were only detected in organic wholegrain flour samples from the UK (3.5 µg/kg) compared with those from Germany (2.1 µg/kg). Mean concentrations which exceeded the MCL for OTA, were only found in conventional white flour and organic wholegrain spelt flour from the UK (Table 4.12.1). However, due to the low numbers of samples collected for each individual flour type in each country the comparisons of 4-way interaction means need to be interpreted with caution.

When the 2-way interaction between farming system and flour type was examined further, concentrations in white flour were found to be similar in both conventional and organic samples, while concentrations in wholegrain flour were significantly (approximately 80%) higher in conventional (2.74 µg/kg) than organic flour samples (1.46 µg/kg) (Table 4.12.2). In terms of comparison between farming systems among the same flour type, concentrations of T-2/HT-2 in white and wholegrain flour were significantly different in conventional, but not organic flour samples (Table 4.12.2).

When the 2-way interaction between wheat type and flour type was examined further, concentrations of T-2/HT-2 in white flour were found to be similar in both spelt and common samples, while concentrations in wholegrain flour were significantly (approximately 100%) lower in spelt (1.25 µg/kg) than in common wheat samples (2.49 µg/kg) (Table 4.12.3). Concentrations of T-2/HT-2 in white and wholegrain flour were significantly different in common but not spelt wheat samples (Table 4.12.3).

When the 2-way interaction between country and flour type for T-2/HT-2 mycotoxins was examined further, concentrations in white flour were found to be similar in both countries, while T-2/HT-2 concentrations in wholegrain flour were significantly (approximately 90%) higher in flour samples from the UK (2.43 µg/kg) than those from Germany (1.27 µg/kg) (Table 4.12.4). When flour types within the same country were compared, concentrations of T-2/HT-2 in wholegrain flour were significantly higher than in white flour in the UK (0.83 µg/kg for white VS 2.43 µg/kg for wholegrain), but not for samples from Germany (0.9 µg/kg for white VS 1.27 µg/kg for wholegrain) (Table 4.12.4).

When year was included as a factor in separate 4-factor ANOVAs for common and spelt wheat, significant differences in (a) T-2/HT-2, ZEA and OTA, but not DON concentrations were detected between common wheat samples collected in 2016 and 2017 (Appendix 4.40), while (b) no significant differences in mycotoxin concentrations could be detected between spelt samples taken in 2015 and 2017 (Appendix 4.41).

Correlation coefficients between phenolic phytochemicals and mycotoxins

Pearson correlation analysis was performed to correlate the concentrations of phenolic phytochemicals and mycotoxins. Only very weak positive linear correlations were found (a) between total antioxidant capacity by ABTS and mycotoxin parameters including DON ($R=0.182$, $R<0.05$), T-2/H-2 ($R=0.156$, $R<0.05$) and total mycotoxins ($R=0.197$, $R<0.05$); also (b) between TPO and ZEA ($R=0.193$, $R<0.05$) (Table 4.13).

Table 4.11 Main effect means ± SE and p-values for the effects of, and interactions between, country (Germany and UK), wheat species (common vs spelt wheat) , farming system (organic vs conventional) and/or flour type (white vs wholegrain) on the % of wheat flour samples testing positive for specific mycotoxins .

Factors	**DON**	**T-2/HT-2**	**ZEA***	**OTA***
Farming system				
Conventional (n=20)	57±8	59±9	86±4	99±1
Organic (n=22)	43±7	63±8	89±4	98±1
Species				
Spelt wheat (n=22)	51±8	64±9	86±4	98±1
Common wheat (n=20)	48±7	58±8	89±4	99±1
Flour type				
White (n=20)	46±7	51±8	86±5	98±1
Wholegrain (n=22)	53±8	70±9	89±3	99±1
Country				
Germany (n=20)	41±7	62±9	88±4	99±1
UK (n=22)	57±8	60±9	87±5	98±1
ANOVA 1 (p-values)				
Main Effects				
Farming System (FS)	NS	NS	NS	NS
Species (SP)	NS	NS	NS	NS
Flour Type (FT)	NS	**0.0014**	NS	NS
Interactions				
FS × SP	NS	**0.0185**	NS	NS
FS x FT	NS	NS	NS	NS
SP x FT	NS	NS	NS	NS
FS x SP x FT	NS	NS	NS	NS
ANOVA 2 (p-values)				
Main Effects				
Farming System (FS)	**0.0467**	NS	NS	NS
Species (SP)	NS	NS	NS	NS
Country (CT)	NS	NS	NS	NS
Interactions				
FS × SP	NS	**0.0058** [1]	NS	**0.0225** [1]
FS × CT	**0.0393** [2]	*0.0685*	*0.0815*	*0.0776*
SP x CT	NS	NS	NS	NS
FS x SP x CT	NS	NS	*0.0825*	NS

* p-values were from analyses performed using cube transformed data, means and SE presented were calculated using non-transformed data;
[1] see Table 4.11.1 for interaction means ± SE; [2] see Table 4.11.2 for interaction means ± SE.

Table 4.11.1 Interactions means ± SE for the effects of farming system (organic vs conventional) and wheat species (Spelt vs common wheat) on the percent of samples testing positive for T-2/HT-2 and OTA.

	Factor 1	**Factor 2 Farming System**	
Parameter	**Species**	**Conventional**	**Organic**
T-2/HT-2	Spelt wheat	55 ±15 B b	72 ± 11 A a
	Common wheat	64 ±12 A a	52 ± 12 B b
OTA*	Spelt wheat	100 ± 0 A a	97 ± 2 A a
	Common wheat	98 ± 2 A a	100 ± 0 A a

For each parameter assessed means labelled with capital letter within the same row or the same lower case letter within the same column are not significant different (Tukey's honestly significant difference test P<0.05)
*Pairwise comparisons of means were carried out on cube transformed data, means and SE presented were calculated without cube transformed data.

Table 4.11.2 Interactions means ± SE for the effects of country (Germany vs UK) and farming system (organic vs conventional) on the percent of samples testing positive for DON

	Factor 1	**Factor 2 Farming System**	
Parameter	**Country**	**Conventional**	**Organic**
DON	Germany	41±10 A b	42± 9 A a
	UK	73±11 A a	44±10 B a

For each parameter assessed means labelled with capital letter within the same row or the same lower case letter within the same column are not significant different (Turkey's honestly significant difference test P<0.05)

Table 4.12 Main effect means ± SE and p-values for the effects of, and interactions between, country (Germany and UK), wheat species (common vs spelt wheat), farming system (organic vs conventional) and flour type (white vs wholegrain) on **mycotoxin concentrations** in wheat flour samples.

	Mycotoxin concentration (µg/kg)			
Factor	**DON***	**T-2/HT-2***	**ZEA***	**OTA***
Farming System				
Conventional (n=181)	60 ±6	2.4 ±0.5	3.7 ±0.3	2.9 ±0.1
Organic (n=171)	49 ±9	2.6 ±0.6	3.8 ±0.3	3.1 ±0.1
Wheat species				
Spelt wheat (n=106)	33 ±5	1.2 ±0.2	3.5 ±0.4	2.6 ±0.1
Common wheat (n= 246)	63 ±8	1.4 ±0.2	3.8 ±0.2	3.2 ±0.1
Flour Type				
White (n=214)	48 ±7	1.7 ±0.4	3.9 ±0.2	2.9 ±0.1
Wholegrain (n=138)	63 ±9	3.8 ±0.7	4.2 ±0.3	3.1 ±0.1
Country				
Germany (n=158)	41 ±6	2.3 ±0.6	4.1 ±0.3	2.3 ±0.1
UK (n=194)	65 ±9	2.7 ±0.5	3.4 ±0.2	3.6 ±0.1
Maximum contamination level (MCL) µg/kg	750[#]	50[##]	75[#]	3[#]
ANOVA results (p-values)				
Main Effects				
Farming System (FS)	**0.006**	NS	NS	NS
Species (SP)	NS	NS	**0.0286**	NS
Flour Type (FT)	*0.0635*	**<0.0001**	NS	NS
Country (CT)	**0.0111**	NS	**0.0483**	**<0.0001**
Interactions				
FS × SP	NS	NS	NS	**0.0051**
FS × FT	NS	**0.0307** [2]	NS	NS
FS × CT	**0.0481**	NS	NS	NS
SP × FT	NS	**0.0163** [3]	NS	NS
SP × CT	NS	NS	NS	**0.0002**
FT × CT	NS	**0.0006** [4]	NS	NS
FS × SP × FT	NS	NS	NS	NS
FS × SP × CT	NS	NS	NS	**0.0392**
FS × FT × CT	**0.0257**	NS	0.0593	NS
SP × FT × CT	NS	NS	NS	NS
FS × SP × FT × CT	**0.0007** [1]	0.0804	NS	**0.0085** [1]

*whose p-values were carried out on log+1 transformed data, means and SE presented were calculated with non-log+1 transformed data; [#] MCL(EC2006); [##] recommended MCL (EC 2006 & 2013)

[1]See Table 4.12.1 for interaction means ± SE; [2]See Table 4.12.2 for interaction means ± SE; [3]See Table 4.12.3 for interaction means ± SE; [4]See Table 4.12.4 for interaction means ± SE

Table 4.12.1 Interactions means ± SE for the effects of country (Germany vs UK), species (spelt vs common wheat), farming system (organic vs conventional) and flour type (white vs wholegrain) on DON and OTA concentrations

			Factor 3 Farming system			
			Conventional		**Organic**	
Mycotoxin	**Factor 1**	**Factor 2**	**Factor 4 Flour Type**			
Parameter	**Country**	**Species**	**White**	**Wholegrain**	**White**	**wholegrain**
			ppb			
DON	Germany	Spelt wheat	40 ±15 A a	65 ±26 A ab	25 ±11 A a	26 ±10 A a
		Common wheat	31 ± 9 AB a	28 ±28 B b	31 ±13 B a	110 ±41 A a
	UK	Spelt wheat	67 ±17 A a	19 ± 9 AB b	15 ± 8 B a	35 ±10 AB b
		Common wheat	65 ±11 B a	116 ±22 A a	79 ±33 BC a	46 ±24 C b
OTA	Germany	Spelt wheat	2.8 ±0.3 AB b	3.0 ±0.3 A ab	2.0 ±0.2 B b	2.1 ±0.2 AB bb
		Common wheat	1.9 ±0.2 B c	1.9 ±0.4 BC c	2.3 ±0.2 AB b	2.7 ±0.3 A ab
	UK	Spelt wheat	3.3 ±0.7 AB ab	2.4 ±0.5 AB bc	2.2 ±0.4 B b	3.5 ±0.5 A a
		Common wheat	3.5 ±0.2 B a	3.7 ±0.3 AB a	4.5 ±0.4 A a	3.7 ±0.3 AB a

For each parameter assessed means labelled with capital letter within the same row or the same lower case letter within the same column are not significant different (Tukey's honestly significant difference test P<0.05);

Pairwise comparisons of means were carried out on log+1 transformed data, means and SE presented were calculated with non-log+1 transformed data.

Table 4.12.2 Interactions means ± SE for the effects of farming system (organic vs conventional) and flour types (white vs wholegrain) on T-2/HT-2 concentrations (µg/kg)

	Factor 1	Factor 2 Flour type	
Parameter	**Farming system**	**White**	**Wholegrain**
T-2/HT-2	Conventional	0.80 ±0.11 B a	2.74 ±0.50 A a
	Organic	0.95 ±0.15 A a	1.46 ±0.20 A b

For each parameter assessed means labelled with capital letter within the same row or the same lower case letter within the same column are not significant different (Tukey's honestly significant difference test P<0.05);

Pairwise comparisons of means were carried out on log+1 transformed data, means and SE presented were calculated with non-log+1 transformed data.

Table 4.12.3 Interactions means ± SE for the effects of species (spelt vs common wheat) and flour types (white vs wholegrain) on T-2/HT-2 concentrations (µg/kg)

	Factor 1	Factor 2 Flour Type	
Parameter	**Species**	**White**	**Wholegrain**
T-2/HT-2	Spelt wheat	1.04±0.20 A a	1.25±0.22 A b
	Common wheat	0.81±0.10 B a	2.49±0.38 A a

For each parameter assessed means labelled with capital letter within the same row or the same lower case letter within the same column are not significant different (Tukey's honestly significant difference test P<0.05);

Pairwise comparisons of means were carried out on log+1 transformed data, means and SE presented were calculated with non-log+1 transformed data.

Table 4.12.4 Interactions means ± SE for the effects of countries (Germany vs UK) and flour types (white vs wholegrain) on T-2/HT-2 concentrations (µg/kg)

	Factor 1	Factor 2 Flour Type	
Parameter	**Country**	**White**	**Wholegrain**
T-2/HT-2	Germany	0.90±0.13 Aa	1.27±0.25 Ab
	UK	0.83±0.14 Ba	2.43±0.36 Aa

For each parameter assessed means labelled with capital letter within the same row or the same lower case letter within the same column are not significant different (Tukey's honestly significant difference test P<0.05);

Pairwise comparisons of means were carried out on log+1 transformed data, means and SE presented were calculated with non-log+1 transformed data.

Table 4.13 Correlation coefficients between phenolic phytochemicals and mycotoxins.

		Total Phenolic content (TPO)	Total Antioxidant capacity		Total Flavonoids	Total Phenolic Components by HPLC
			FRAP	ABTS		
Mycotoxin	DON	0.025	0.108	**0.182****	0.054	0.063
	ZEA	**0.193****	0.061	-0.087	-0.080	0.130
	OTA	-0.090	-0.038	0.047	0.043	0.012
	T-2/HT-2	0.043	0.039	**0.156***	-0.006	0.045
	Total mycotoxins	0.039	0.114	**0.197****	0.050	0.075

*,** were significant at 0.05, 0.01 probability level, respectively

4.6.2 Discussion

A wide range of cohort studies have shown associations between the consumption of wholegrain flour products and a reduced incidence of type-2 diabetes, cardiovascular diseases and certain cancers (e.g. colorectal, pancreatic and gastric cancer). Health benefits have been linked to the substantially higher fibre, mineral, vitamin and (poly)phenol/antioxidant intake with wholegrain compared with polished grains or white flour based cereal products (Jones and Engleson, 2010).

However, concerns have been raised about the safety of wholegrain consumption in terms potentially increasing exposure to mycotoxins, toxic metals and acrylamide (Thielecke and Nugent, 2018) and especially mycotoxin contamination of cereals continues to be serious threat to public health globally (Bui-Klimke and Wu, 2015).

In the study reported here, the mean concentrations of the *Fusarium* mycotoxin DON, T-2/HT-2 and ZEA in wholegrain and white flour brands from both Germany and the UK were all more than 10 times lower than the maximum contamination levels (MCL) set by the EU (Table 4.12). Although wholegrain flour had higher mean concentrations of T-2/HT-2, the overall very low mycotoxin loads suggests that both wholegrain and white flour consumption do not pose a health risk to consumers.

In contrast, mean concentrations of OTA were 20% higher than the MCL in samples from the UK and only around 20% lower than the MCL in samples from Germany, but overall were very similar in wholegrain and white flour (Table 4.12). It should be pointed out, that the commercial ELISA test system used in the current analysis is not as accurate as HPLC-based mycotoxin quantification methods, and although concentrations were only marginally higher than the MCL, the lack of a “safety-margin” between the mean concentrations detected and the MCL set by the EU should still be viewed with caution. This is important because Ochratoxin A (OTA) has been shown to have nephrotoxic and immunosuppressive effects, and is suspected to also have carcinogenic and teratogenic effects at low concentrations in all experimental animal systems used in tests (European Food Safety Authority, 2006).

Therefore, the discussion of the differences between wholegrain and white flour produced in different countries (UK vs Germany), from different wheat species (spelt vs common wheat) and with different agronomic protocols (organic vs conventional) has related differences to results from experimental and farm survey based studies that focused on identifying climatic, crop genetic, agronomic, grain processing and quality assurance related parameters affecting mycotoxin levels.

Effect of farming system

Apart from climatic conditions during the growing season (especially after tillering) and at harvest, the level of mycotoxins contamination in cereals is affected by (b) agronomic management factors including crop protection, tillage, fertilisation, rotation design/pre-crop and variety choice (Dill-Macky and Jones, 2000; Krebs *et al.*, 2000; Heier *et al.*, 2005; Supronienė *et al.*, 2012) and (c) postharvest management practices (e.g. drying and cleaning of harvested grain and storage conditions) (Magan and Olsen, 2004).

Fungicides are widely used in conventional cereal production to control foliar diseases (including *Fusarium* head blight), but are prohibited under organic farming standards. It has therefore been argued that organic cereal crops are at a higher risk from fungal diseases and mycotoxin contamination (Trewavas, 2001a). However, this claim has not been substantiated (Bernhoft *et al.*, 2012; Brodal *et al.*, 2016a) and studies into the effect of fungicides application on *Fusarium* head blight severity, *Fusarium* grain infection and mycotoxin levels have often shown variable and/or contradictory results (Magan *et al.*, 2002; Heier *et al.*, 2005). There is also evidence that the use of fungicides may increase mycotoxin production (e.g. due to stress imposed on the fungal pathogen (Simpson *et al.*, 2001; Ellner, 2005; Köpke *et al.*, 2007; Mankevičienė *et al.*, 2008; Audenaert *et al.*, 2010). In the study reported here organic wheat flour, overall, had 20% lower DON but similar contamination levels of T-2/HT-2, ZEA and OTA (Table 4.12).

For DON these results are consistent with a range of qualitative reviews (Benbrook, 2005; Gottschalk *et al.*, 2007; Brodal *et al.*, 2016b) and the only previous meta-analysis of comparative DON contamination data (Smith-Spangler *et al.*, 2012a) which reported significantly lower levels of DON in organic compared with conventional common wheat samples (SMD, -0.94 [CI, -1.27 to -0.62]; $P<0.01$; $I^2=63$).

However, contrasting effects of farming systems on DON contamination were detected between countries and/or flour types found in the study reported here (Table 4.12). For example, substantial differences in DON concentrations were detected for (a) wholegrain common wheat flour in Germany (approximately 4 times higher in organic flour), (b) white spelt flour in the UK (approximately 4 times higher in conventional flour), (c) wholegrain common wheat flour in the UK (approximately 2 times higher in conventional flour) (Table 4.12.1). This variation could be due to differences in (a) agronomic protocols used for spelt and common wheat and/or (b) both agronomic protocols used and pre-harvest climatic conditions between the UK and Germany, since the degree of contamination with *Fusarium* mycotoxins is determined primarily by agronomic parameters (e.g. rotation design, tillage, fertilisation and crop protection regimes) and environmental conditions before harvest. For example, Paulsen

and Weißmann (2002a) identified 13 agronomic/farm management factors that may affect mycotoxin formation and contamination in food and feed crops, which may also explain differences in *Fusarium* mycotoxin contamination between organic and/or conventional crop farming systems.

For OTA, the results reported here (Table 4.12) contradict a range of studies carried out in the early 2000s which found significantly higher OTA levels in organic cereals or cereal products. Limited access to grain drying facilities and poor on-farm storage conditions were described as the main reasons for the higher OTA levels in organic wheat/cereal products at that time (Lund and Frisvad, 2003; Köpke *et al.*, 2007; Magan and Aldred, 2007). OTA contamination levels are mainly determined by climatic conditions during harvest and post-harvest treatment (especially drying to reduce grain moisture to levels) and storage conditions which prevent infection growth and mycotoxin production by common mould fungi (*Aspergillus* and *Penicillum* spp.) (Magan and Aldred, 2007; Nleya *et al.*, 2018). However, the results here are consistent with the findings of a more recent meta-analysis of studies which compared mycotoxin contamination in organic and conventional cereals, which reported that OTA levels in organic cereals/cereal products have decreased over time and are now similar to those found in conventional cereals (Juan Wang *et al.*, 2019). These improvements are thought to be mainly due to improved access to state-of-the-art grain drying and storage facilities and better post-harvest quality assurance (Juan Wang *et al.*, 2019).

Effect of wheat species

Spelt wheat (*T. spelta*), unlike most other cereals including common wheat, has husks/glumes and a range of studies have concluded that the husks act as physical barrier that reduces fungal colonisation and mycotoxin production in wheat grains/kernels (Foroud and Eudes, 2009; Mankeviciene *et al.*, 2014). This is consistent with results presented here which found (a) overall lower ZEA concentrations in spelt compared with common wheat flour (Table 4.12), (b) lower T-2/HT-2 concentrations in wholegrain spelt flour than wholegrain common wheat (Table 4.12.3), (c) lower DON concentrations in conventional wholegrain spelt flour than in conventional wholegrain common wheat flour in the UK (Table 4.12.1) and (d) lower OTA concentrations in conventional, wholegrain spelt flour than common wheat, and organic, white spelt than common wheat flour in the UK (Table 4.12.1).

There is also a range of other studies which reported that the relative resistance to mycotoxin producing fungi is higher in spelt than other wheat species (Tanaka *et al.*, 1988; Döll *et al.*, 2000; Foroud and Eudes, 2009; Mankeviciene *et al.*, 2014; Rachoń *et al.*, 2016; Góral and Ochodzki, 2017). For example, a study by Rachoń *et al.* (2016) compared *Fusarium*

mycotoxin (including DON, T-2, ZEA) concentrations in common, durum, spelt, einkorn and spelt winter-wheat genotypes and showed that spelt wheat had the lowest mycotoxin levels. It should be pointed out that most spelt wheat varieties also have longer stems/straws than modern common wheat varieties grown in Europe, which is also thought to reduce the risk of grain infections by *Fusarium* spp. (Longin and Würschum, 2014).

However, it is important to consider that the mycotoxin concentrations found in flour are determined by three main factors or critical control points (CCPs). These are the (a) climatic and agronomic parameters during the growth and harvest of cereals in the field, (b) post-harvest treatments (e.g. drying) and storage and (c) mycotoxin testing-based quality assurance (QA) protocols used by seed storage/marketing/processing companies (Magan and Olsen, 2004). Although the SBB results reported provide an overall measure of the levels of mycotoxin exposure from spelt and common wheat experienced by consumers, they do not allow results to be linked to differences in these three CCPs. More detailed farm survey-based approaches which monitor mycotoxin levels and background conditions along the supply chain (from field to supermarket shelf) would therefore be required to explain differences found between spelt and common wheat flour.

Effect of flour type

Milling practices that remove the outer layers of the grain are described as one way to minimise dietary exposure to mycotoxins (Cheli *et al.*, 2013; Zhang *et al.*, 2019).

In the survey reported here, significantly higher mycotoxin loads in wholegrain than white flour were only detected for one *Fusarium* mycotoxin (T-2/HT-2), but T-2/HT-2 concentrations in wholegrain flour were still more than 12 times lower than the MCL (Table 4.12).

The very low levels of contamination with *Fusarium* mycotoxins and very similar OTA levels detected in wholegrain and white flour suggest that there is no substantial difference in health risk associated with consumption of white or wholegrain flours in the UK and Germany. Based on the results of this study there is no justification for changing the current dietary recommendations to increase wholegrain consumption (Bartlomiej *et al.*, 2012; Borneo and Len, 2012; Cho *et al.*, 2013; Thielecke and Nugent, 2018). However, the finding that mean OTA concentrations were above the MCL for 6 out of 8 flour types in the UK and only slightly lower than the MCL for the 2 other UK and all German flour types should be viewed with some concern. While cereal and cereal products are the main dietary source for *Fusarium*-mycotoxins e.g. DON, ZEA, T-2/HT-2), a wider range of foods, including pulses, coffee, cacao, grape juice, dry vine fruits, wine, nuts and spices can significantly contribute to dietary OTA intake (EFSA, 2006). According to the most recent EFSA opinion (EFSA, 2006) there is strong evidence for "*site specific renal toxicity as well as DNA damage and genotoxic effects of OTA*"

and some epidemiological evidence *"for distinct renal diseases and otherwise rare tumours of the kidneys in certain endemic regions of the Balkan Peninsula"*. EFSA reports the lowest observed adverse effect level (LOAEL) as being 8µg/kg body weight per day and applied a composite uncertainty factor of 450 when setting the tolerable weekly intake (TWI) at 120 ng/kg body weight for OTA (EFSA, 2006). Dietary exposure of adult European consumers to OTA is estimated to range from 15 to 60 ng OTA per kg body weight per week which is therefore well below the TWI set by EFSA (EFSA, 2006).

High OTA levels in cereal grains and flour have often been associated with delayed or inefficient grain drying post- harvest and/or poor storage facilities (Birzele *et al.*, 2000; Halstensen *et al.*, 2004; Magan and Aldred, 2005; Magan and Aldred, 2007). Further reductions in OTA levels in wheat flour may therefore come from a detailed review and improvements of postharvest critical control points, including the quality assurance protocols (e.g. the OTA concentrations set as thresholds during testing) by grain storage/marketing companies and/or millers.

It is interesting to note that higher T-2/HT-2 concentrations in wholegrain than white flour were only detected in UK but not Germany (Table 4.12.4), which may be explained by the differences in climatic conditions and/or contrasting *Fusarium* species profiles in the UK and Germany especially during the period before grain harvest (Bernhoft et al. 2012). It is well established that the *Fusarium* species and mycotoxin profiles associated with *Fusarium* head blight (FHB) in wheat and other small grain cereals differ considerably between climatic zones in Europe and that mycotoxin production is affected by environmental conditions especially temperature and rain/humidity (Bottalico and Perrone, 2002; Bernhoft *et al.*, 2012). For example, wet/humid and warm conditions before harvest were shown to significantly increase both HT-2/T-2 contamination in barley, oats and wheats, and lower temperature before grain harvest was found to increase DON concentrations (Bernhoft *et al.*, 2012). The types/profiles of mycotoxins produced on cereal grains differs between *Fusarium* species (Bottalico and Perrone, 2002; Kokkonen *et al.*, 2010). For example, the most frequently found mycotoxins associated with FHB (DON and ZEA) are produced primarily by the pathogenic species *Fusarium graminearum* and *F. culmorum*. In contrast, the occurrence of T-2/HT-2 toxin, which is considered to be substantially more toxic than DON, has been linked mainly to sporadic epidemics of *F. sporotrichioides* and *F. langsethiae* (previously classified as *F. poae*) which are thought to be less plant pathogenic (Bottalico and Perrone, 2002). These *Fusarium* species may therefore mainly infect the outer layers of the grain and not the endosperm, which would explain the lower concentrations in white flour in the UK. Contrasting T-2/HT-2 levels may also have been due to differences in *Fusarium* disease pressure and grain infection levels (Edwards et al. 2011).

Correlation coefficients between phenolic phytochemicals and mycotoxins

Since (a) high concentration of antioxidants including phenolics are known to be produced by plants in response to abiotic (e.g. wounding and heat, water and nutrient stress) and biotic (pest attacks and disease) stress (Nicholson and Hammerschmidt, 1992; Bennett and Wallsgrove, 1994; Almuayrifi, 2013; Baranski *et al.*, 2014); and (b) mycotoxins are secondary metabolites when affected by fungal/disease. The correlation between concentrations of phenolic phytochemicals and mycotoxin has been reported to be significant and positive (Reid *et al.*, 1999). However, this was not consistent with the result of this study. The different response observed could be explained by the confounding effect of environmental conditions, agronomic practices and susceptibility of the genotype which cannot be clearly identified in the SBS samples analysed in this study (Reid *et al.*, 1999).

Daniel *et al.* (1999) found that pesticide application affected the phenolic content in plants; herbicides reducing or decreasing the content of secondary compounds in plants. The application of fungicide also affected the mycotoxin accumulation. However, since information on location of farms, crop varieties and specific agronomic practices used by organic and conventional farmers and post-harvest drying, storage, cleaning, and milling protocols used for organic and conventional grains were not available for the samples analysed here, it was not possible to explore to what extent these parameters affected the quality outcomes measured. In order to explore these possible effects further, smaller on-farm or greenhouse studies would be required where growing conditions and levels of fungal infection could be more tightly controlled.

Potential limitations of the ELISA based analysis method used in the flour survey

As pointed out in the Methods section, standard commercial ELISA-based test kits were used for mycotoxin analysis in the study reported here. These test kits are used widely as part in quality assurance by grain storage and processing companies, but have higher detection limits and are considered less accurate for determining mycotoxin concentrations than other mycotoxin assessment methods (e.g. HPLC or GC mass-spectroscopy-based assays). However, due to the high cost of HPLC, HPLC-MS or GC-MS based analyses and the large number of samples that needed to be examined to identify potentially confounding effects of country, flour type and wheat species we used the commercial ELISA-based test kits in this study. Results show that the method used was sufficiently sensitive to identify both main effects and interaction between factors. However, more sensitive analytical protocols should be considered in future experiments into the effects of pedoclimatic conditions, wheat species/genotype, flour types and farming systems, to quantify mycotoxin levels in cereals more accurately, in particular where the mycotoxin concentrations are either very low or are close to the MCL (Nuryono *et al.*, 2005).

4.6.3 Conclusion

Results from the SBS show that overall, there is no increased risk of dietary mycotoxin exposure from organic wheat flour as suggested in the past (Trewavas, 2001a). In fact, DON concentrations were on average over all flour types slightly (22%), but significantly higher in conventional wheat flours, and in the UK nearly twice as many conventional than organic flour samples tested positive for DON. However, given that DON concentrations were more than 12 times lower in the conventional and organic samples than the MCL this is highly unlikely to be of nutritional relevance.

The results of the SBS study also show that wholegrain flour and white flour has similar concentration of DON, ZEA and OTA, and though there were significant differences for T-2/HT-2, the concentration of T-2/HT-2 in wholegrain and white flour was more than 12 times lower than the MCL set by the EU. This clearly suggests that in the UK and Germany (a) there is no difference in mycotoxin-related health risks between white and wholegrain wheat consumption and (b) concerns about mycotoxin loads should not restrict nutritional recommendations in these countries to switch from white to wholegrain consumption, given the increasing evidence for health benefits of wholegrain (McRae M. 2017).

However, the finding that OTA concentrations were close to, and for a substantial number of samples above the current MCL set by the EU, should be of concern and lead to a critical re-examination of the critical control points pre- and post-harvest (e.g. drying, storage, mycotoxin testing) where OTA contamination can be minimised.

4.7 Effect of harvest year, species, farming system and flour type on pesticide residues of common and spelt wheat flour- shopping basket survey in the UK and Germany

4.7.1 Results

In 2016, flour samples were analysed for 57 different pesticide active ingredients (See Appendix 4.43). In 2017, flour samples were analysed for 492 pesticide active ingredients (See Appendix 4.44). 47 pesticides were analysed in both year (See Appendix 4.42).

In the pesticide assessment, (a) the mean concentration of pesticides in flour samples, and (b) the percentage of samples testing positive for each pesticide contamination, were analysed.

Results from samples taken in 2 years (2016 and 2017) were available, but no samples of white spelt flour could be found in Germany in 2016. It was therefore not possible to include all experimental factors (country, species, farming system and flour type) in the same ANOVA. For data on (a) the proportion of positive samples (= samples in which pesticides residue above the detection limit of the analytical methods used were detected) and (b) the concentrations of pesticide residues were analysed. Therefore two separate 3-factor ANOVAs were carried out with (a) wheat species (common vs spelt wheat), farming system (organic vs conventional) and flour types (wholegrain and white) (ANOVA 1) (with year and country as random factors/replicates) and (b) country (UK vs Germany), species (common vs spelt) and farming system (organic vs conventional) (ANOVA 2) (with year and flour type as random factors/replicates).

When analysing the mean concentrations of pesticides, the concentration in negative samples (= samples in which no pesticides residue was detected by the analytical methods used) were set at half the concentration of the limit of detection of the analytical method as recommended in previous studies (Pussemier *et al.*, 2006; Gottschalk *et al.*, 2007).

In 2016, eight pesticides were detected among 110 samples including deltamethrin, chlormequat, piperonyl butoxide, pirimiphos methyl, alpha-cypermethrin, pendimethanil, tebuconazole, and mepiquat. In 2017, seven pesticides were detected among 147 samples including deltamethrin, chlormequat, piperonyl butoxide, pirimiphos methyl, 2-phenylphenol, glyphosate, and chlorpyrifos methyl.

Proportion of flour samples testing positive for pesticide residues

The only crop protection product (CPP) residues detected in a substantial number of flour samples were chlormequat (detected in 35 % of flour samples) and piperonyl butoxide (detected in 20% of flour samples) (Table 4.14). Other pesticides were detected in a smaller proportion flour samples and included glyphosate (detected in 14% samples), 2-phenylphenol (detected in 5% samples) and alpha-cypermethrin (detected in 13% samples) , and pesticides

found in only very few samples included pirimiphos-methyl (detected in 4% samples) , deltamethrin (detected in 4% samples), mepiquat (detected in 2% samples), pendimethanlin (detected in 2% samples,), tebuconazole (detected in 2% samples), and chlorpyrifos methyl (detected in 1% samples). Only alpha-cypermethrin was detected in more organic than conventional flour samples (25 and 9 samples respectively of 113 samples tested).

Significant main effects were detected only for farming system (Table 4.14). The percentage of flour samples testing positive for (a) at least 1 CPP, (b) multiple (2 or more) CPP residues, (c) chlormequat, and (d) pirimiphos methyl were significantly higher in conventional compared with organic flour samples (Table 4.14). Approximately 3-times more conventional than organic flour samples were found to be contaminated with at least 1 CPP and there were 8 times more conventional than organic samples with multiple CPP residues (Table 4.14).

For piperonyl butoxide a significant interaction between wheat species and farming system was detected. For Spelt wheat, twice the number of positive samples were found in conventional than organic samples (41% vs 22%) but the difference was not significant. For common wheat, ten times more positive samples were found in conventional than organic wheat (30% vs 3%) and the difference was significant (Table 4.14.1).

For chlormequat a significant interaction between country and farming system was detected. The difference in the proportion of positive samples between organic and conventional flour was greater in the UK (5% vs 91 %) than in Germany (0 vs 47%) (Table 4.14.2).

Concentrations of pesticide residues

Sufficient numbers of samples with quantifiable concentrations of individual CPPs to obtain normally distributed data for ANOVA were only available for chlormequat. As a result, factorial ANOVAs to compare the concentrations proportion of positive samples between wheat species, countries, farming systems and flour types could only be carried out for chlormequat and the mean total concentration of CPPs in samples (Table 4.15).

For both chlormequat and total CPP concentrations, significant main effects were detected for all four factors included in the two 3-factor ANOVAs carried out (Table 4.15). Significantly higher concentrations of chlormequat and total CPP residues were found in the UK than in Germany; in common wheat than in spelt wheat; in conventional than in organic and in wholemeal than in white flour samples (Table 4.15). Chlormequat accounted for just under half of the total detectable CPP residue concentrations and mean concentrations were 2 orders of magnitude lower than the MRL (Table 4.15).

For total CPP residues, ANOVA also detected significant two-way interactions between (a) wheat species and farming system and (b) wheat species and country (Table 4.15). Higher

concentrations of total CPP residues in common wheat than in spelt wheat were only detected in conventional, but not organic flour samples (Table 4.15.1). Also higher concentrations of total CPP residues in common wheat than in spelt wheat were only detected in Germany, but not the UK (Table 4.15.4).

For both chlormequat and total CPP residues, ANOVA detected significant two-way interactions between (a) flour type and farming system, (b) country and farming system (Table 4.15). Significantly higher concentrations of chlormequat and total CPP residues in wholemeal flour than in white flour were only detected in conventional, but not organic flour samples (Table 4.15.2). Also, higher concentrations of both chlormequat and total CPP residues in UK flours than in German flour samples were only detected in conventional, but not organic flour samples (Table 4.15.3).

Table 4.14 Main effect means ± SE and p-values for the effects of, and interactions between, country (Germany and UK), wheat species (common vs Spelt wheat) , farming system (organic vs conventional) and flour type (white vs wholemeal) on the % of wheat flour samples testing positive for specific crop protection product (CPP), at least 1 CCP and multiple (2 or more) CCP residues.

Factor	chlormequat	piperonyl butoxide*	2-phenyl-phenol [##]	glyphosate [##]	alpha-cypermethrin [###]	at least 1 CPP	Multiple CPP residues
Country							
Germany (n=16)	23±11	27±9	13±13	4±3	14.6±9.7	54±11	15±6
UK (n=15)	45±12	20±6	0	16±12	20.6±9.4	57±11	24±9
Species							
Spelt wheat (n=15)	33±12	31±9	0	4±3	25±10	64±10	13±6
Common wheat (n=16)	35±11	17±5	13	16±12	11±8	47±11	24±9
Farming system							
Conventional (n=15)	68±11	36±9	13±13	21±11	9±9	87±6	36±9
Organic (n=16)	3±2	12±5	0	0	25±9	25±8	4±2
Flour type							
White (n=15)	31±11	20±8	13±13	7±5	7±7	49±11	14±7
Wholemeal (n=16)	37±12	27±7	0	14±11	26±10	61±10	24±8
ANOVA 1 (p-values)							
Main Effects							
Farming System (FS)	**0.003**	**0.0438**	-	-	-	**0.002**	**0.0116**
Species (SP)	NS	NS	-	-	-	NS	NS
Flour type (FT)	NS	NS	-	-	-	NS	NS
Interactions	NS	NS	-	-	-	NS	NS
ANOVA 2 (p-values)							
Main Effects							
Farming System (FS)	**<0.0001**	**0.0045**	-	-	-	**<0.0001**	**0.0028**
Species (SP)	NS	NS	-	-	-	NS	NS
Country (CT)	NS	NS	-	-	-	NS	NS
Interactions							
SP × FS	NS	**0.04223** [1]	-	-	-	NS	NS
CT × FS	**0.03401** [2]	NS	-	-	-	NS	NS

[#], only assessed in 2016; [##] assessed in both 2016 and 2017, but data shown are for 2017 only, since there were no positive samples in 2016.
[###] assessed in both 2016 and 2017, but data shown are for 2016 only, since there were no positive samples in 2016.
[1] see Table 4.13.1 for Interaction means ± SE; [2] see Table 4.13.2 for Interaction means ± SE ;
* p-values are for ANOVAs carried out on log+1 transformed data, means and SE presented were calculated with non-log+1 transformed data

Table 4.14.1 Interactions means ± SE for the effects of species and farming systems on percentage of positive samples detected with piperonyl butoxide.

	Factor 1	Factor 2 Farming System	
Crop protection products (CPPs)	**Species**	**Conventional**	**Organic**
piperonyl	Spelt Wheat	41±17 A a	22±9 A a
butoxide	Common Wheat	30 ± 7 A a	3 ±2 B b

For each parameter assessed means labelled with capital letter within the same row or the same lower case letter within the same column are not significant different (Tukey's honestly significant difference test P<0.05);

Table 4.14.2 Interactions means ± SE for the effects of country and farming systems on percentage of positive samples detected with chlormequat.

	Factor 1	Factor 2 Farming System	
Crop protection products (CPPs)	**Country**	**Conventional**	**Organic**
Chlormequat	Germany	47±18 A b	0±0 B a
	UK	91± 6 A a	5±3 B a

For each parameter assessed means labelled with capital letter within the same row or the same lower case letter within the same column are not significant different (Tukey's honestly significant difference test P<0.05);

Table 4.15 Main effect means (± SE) and p-values for the effects, and interaction between, country, cereals species, farming system and flour type on concentrations of chlormequat and all crop protection products in wheat flour samples collected between 2016 and 2017.

	concentration (µg/kg)	
Factor	**Chlormequat***	**Total CPP residues* #**
Farming system		
Conventional (n=125)	55 ± 5.0	110 ± 11
Organic (n=135)	5 ± 0.3	26 ± 0
Species		
Spelt wheat (n=85)	16 ± 3.0	39 ± 3
Common wheat** (n=175)	36 ± 3.8	80 ± 9
Flour type		
White (n=151)	22 ± 2.0	54 ± 4
Wholemeal (n=109)	40 ± 6.0	84 ±13
Country		
Germany (n=121)	24 ± 4.4	54 ± 6
UK (n=139)	34 ± 3.6	77 ±10
Maximum residue level (MRL) µg/kg (EC 2018)	4000	No MRL
ANOVA 1-results (p-values)		
Main Effects		
Species (SP)	**0.0007**	**0.0001**
Farming System (FS)	**<.0001**	**<.0001**
Flour Type (FT)	**0.0114**	**0.0002**
Interactions		
FS × SP	NS	**0.0037** [1]
FS × FT	**0.0008** [3]	**0.0007** [3]
SP × FT	NS	NS
SP × FS × FT	NS	*NS*
ANOVA 2-results (p-values)		
Main Effects		
Farming System (FS)	**<.0001**	**<.0001**
Species (SP)	**0.0006**	**0.0003**
Country (CT)	**<.0001**	**<.0001**
Interactions		
FS × SP	NS	**0.0393**
FS × CT	**<0.0001** [4]	**0.0021** [4]
SP × CT	NS	**0.0331** [2]
FS × SP × CT	*0.0963*	*0.0662*

*after reciprocal (1/x) transfroamtion

alpha-cypermethrin and glyphosate were not included in the sum because alpha-cypermethrin data only been available for 2017 and the detection limits for glyphosate having been different in the analytical methods used 2016 and 2017.

[1] see Table 4.14.1 for interaction means ± SE; [2] see Table 4.14.2 for interaction means ± SE; [3], see Table 4.14.3 for interaction means ± SE; [4], see Table 4.14.4 or interaction means ± SE

Table 4.15.1 Interactions means ± SE for the effects of wheat species and farming systems on all crop protection products concentration (µg/kg).

Crop protection products (CPPs)	**Factor 1**	**Factor 2 Farming System**	
	Species	Conventional	Organic
Total CPPs	Spelt wheat	60 ± 7 A b	27 ± 1 B a
	Common wheat	128 ±15 A a	26 ±0.4 B a

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (P<0.05). Pairwise comparisons of means were carried out on reciprocal transformed data; means and SE presented were calculated with non-reciprocal transformed data.

Table 4.15.2 Interactions means ± SE for the effects of flour type and farming systems on chlormequat and all crop protection products concentration (µg/kg).

Crop protection products (CPPs)	**Factor 1**	**Factor 2 Farming System**	
	Flour Type	**Conventional**	**Organic**
Chlormequat	**White**	36 ± 4 A b	5 ±0.4 B a
	Wholemeal	88 ±11 A a	5 ±0.5 B a
Total CPPs	**White**	79 ± 7 A b	26 ±0.4 B a
	Wholemeal	166 ±28 A a	27 ± 1 B a

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (P<0.05). Pairwise comparisons of means were carried out on reciprocal transformed data, means and SE presented were calculated with non-reciprocal transformed data.

Table 4.15.3 Interactions means ± SE for the effects of country and farming systems on chlormequat and all crop protection products concentration (µg/kg).

Crop protection products (CPPs)	Factor 1	Factor 2 Farming System	
	Country	**Conventional**	**Organic**
Chlormequat	**Germany**	46 ±9 A b	5 ±0 B a
	UK	62 ±6 A a	6 ±1 B a
Total CPPs	**Germany**	88 ±10 A b	25 ±0 B a
	UK	128 ±19 A a	27 ±1 B a

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (P<0.05). Pairwise comparisons of means were carried out on reciprocal transformed data, means and SE presented were calculated with non-reciprocal transformed data.

Table 4.15.4 Interactions means ± SE for the effects of country and wheat species on total crop protection products concentrations (µg/kg).

Crop protection products (CPP)	Factor 1	Factor 2 Country	
	Wheat species	Germany	UK
Total CPPs	Spelt wheat	35 ±4 B b	46 ± 6 A a
	Common wheat	69 ±9 B a	87 ±13 A a

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (P<0.05). Pairwise comparisons of means were carried out on reciprocal transformed data, means and SE presented were calculated with non-reciprocal transformed data.

4.7.2 Discussion

The CPP residues present at detectable levels in wheat flour were (a) the plant growth regulators chlormequat and mepiquat (both quaternary ammonium compounds), (b) glyphosate (an organophosphorus herbicide and crop desiccant), (c) pendimethalin (a dinitroaniline herbicide), (d) chlorpyrifos-methyl and pirimiphos methyl (both organophosphorus insecticide), (e) deltamethrin and alpha-cypermethrin (which are both synthetic pyrethroid insecticide), (f) piperonyl butoxide (a pesticide synergist which is included in many pyrethrum or synthetic pyrethroid insecticide-based CPPs; it enhances the activity of insecticides by inhibiting insect enzymes which break down insecticides), (g) tebuconazole (a triazole fungicide) and (h) 2-phenylphenol (a fungicide usually used post-harvest, e.g. for disinfection of surfaces and seed boxes) (NPIC, 2019) and are all permitted for use in the EU (Table 4.14). Also, the concentrations of residues of CPPs detected in wheat flour samples were all below the maximum residue levels (MRLs) set by the EU for the respective individual active ingredients detected (Table 4.15) (European Commission, 2018).

Although residues below the maximum residue level (MRL) set for specific active ingredients in CPPs are considered safe by regulators, there is growing concern about negative public health impacts of chronic dietary exposure to below MRL-levels of CPPs (Blair *et al.*, 2015; Nicolopoulou-Stamati *et al.*, 2016). Concerns about chronic dietary exposure to CPPs focuses on the realisation that (a) consumers are exposed to mixtures of CPPs in food, while regulatory safety evaluations only require toxicity testing of individual active compounds (Hernández *et al.*, 2013; Kjeldsen *et al.*, 2013) and (b) many of the most widely used CPPs were shown to be endocrine disrupting chemicals (EDCs) (Mnif *et al.*, 2011). Like hormones, EDCs can have physiological impacts at very low concentrations and there is growing evidence from both *in vitro* and animal studies demonstrating additive effects of exposure to mixtures of EDCs (Heindel and vom Saal, 2009; Schug *et al.*, 2011; Yang *et al.*, 2015; Petrakis *et al.*, 2017).

Nearly all CPPs detected in wheat flour (chlormequat, chlorpyrifos-methyl, glyphosate, phenyl-phenol, piperonyl butoxide, cypermethrin, deltamethrin, tebuconazole) are suspected or confirmed endocrine disrupting chemicals (EDC) (Mnif *et al.*, 2011; EPA, 2015; European Food Safety Authority, 2015).

EDCs can have activity at very low doses and have U-shaped or inverted U-shaped, non-monotonic dose-response curves. This makes it virtually impossible to detect their physiological impacts in the animal (usually rodent) model-based toxicity tests required as part of the regulatory approval process (Heindel and vom Saal, 2009; Schug *et al.*, 2011; Yang *et al.*, 2015; Petrakis *et al.*, 2017). Also, exposure to ECDs can result in epigenetic alterations and genetic programming which may eventually lead to an increased incidence of a variety of

diseases (including obesity, type-2 diabetes, immune abnormalities and cancer) later in life or in subsequent generations (Heindel and vom Saal, 2009; Schug *et al.*, 2011; Xin *et al.*, 2015; Yang *et al.*, 2015; Petrakis *et al.*, 2017). However, epigenetic effects of ECDs can only be accurately identified by extensive, multigenerational animal studies, which are also not required as part of standard regulatory pesticide safety testing protocols (Matthiessen *et al.*, 2017).

Effect of farming system

Data from this study suggest that switching to organic cereal production methods will result in a 10-fold reduction in CCC, a 4-fold reduction in total CPP residues and an 8-fold reduction in the number of wheat samples contaminated with multiple CPP residues (Tables 4.14 and 4.15). Considering that (a) cereal products account for between a quarter (e.g. in Germany and the UK) and half (e.g. in China and India) of daily calorie intake (National Geographic, 2019) and (b) most of the CPPs detected in wheat were suspected or confirmed EDCs, switching to organic cereal products could well have significant positive health impacts. Recent epidemiological studies from the US suggest that this may particularly apply to reproductive health, since reduced dietary intake of CPPs via fruit and vegetables was reported to be linked to higher sperm quality in men (Chiu *et al.*, 2015) and higher pregnancy rates among women undergoing infertility treatment (Chiu *et al.*, 2018). However, dietary exposure to EDCs have also been linked to attention deficit disorder (ADHD), Parkinson's and Alzheimer's disease, obesity, diabetes, hypospadias and cancer (Bjørling-Poulsen *et al.*, 2008; Burns *et al.*, 2013; Zaganas *et al.*, 2013; Yang *et al.*, 2015; Petrakis *et al.*, 2017).

The most frequently detected CPP and the highest residue levels (55 µg/kg in conventional and 5 µg/kg in organic flour, table 4.14) were detected for CCC, which accounted for approximately half the total detected CPP residue concentration in conventional wheat but only a quarter of the CPP concentration in organic wheat. CCC is used in cereals production to reduce stem length/longitudinal shoot growth and thereby increasing lodging resistance and improving harvest index and yield (EFSA, 2008; FAO, 2017). The MRL set by the EU for chlormequat is 4 mg/kg which ~100 time higher than the concentrations found in flour samples in this study. However, long-term professional exposure to CCC was described to increase the risk of liver damage, tumours, and reduced reproductive and foetal health and fertility in animal models, pigs and/or humans (Sørensen and Danielsen, 2006; EFSA, 2010; LI *et al.*, 2011; Nisse *et al.*, 2015; Huang *et al.*, 2016). The Danish pig industry recommended limiting the use of grain from crops treated with chlormequat and other growth regulators for breeding stock in 1990 and evidence that CCC negatively affects fertility in experimental animals at concentrations that are below the acceptable intake emerged more than 10 years ago

(Sørensen and Danielsen, 2006). However, CCC is still permitted for use in cereal crops for human consumption in most EU-countries.

The 4-fold reduction in dietary CPPs intake and lower exposure to multiple CPPs with organic cereals (Table 4.15) may also partially explain the results of recent cohort studies comparing health outcomes in individuals with low and high levels of organic food consumption. These studies reported significant positive associations between high levels of organic food consumption and lower risks of obesity, metabolic syndrome, pre-eclampsia and eczema, hypospadias and cancer (Kesse-Guyot *et al.*, 2013; Bradbury *et al.*, 2014; Torjusen *et al.*, 2014; Brantsaeter *et al.*, 2016; Baranski *et al.*, 2017; Baudry *et al.*, 2018).

The finding of a higher proportion of organic (25%) than conventional (5%) flour samples and the high testing positive for residues of alpha-cypermethrin residues should be further investigated (Table 4.14). This and the finding of piperonyl-butoxide (an activity enhancer often use in pyrethroid formulations) in 12% of organic flour samples may indicate the use of synthetic pyrethroid products in organic farming systems (Table 4.14). Different to pyrethrum-CPPs (which are based on plant extract-based “natural” pyrethrins as active ingredients), synthetic pyrethoid-based CPPs are not approved for use in organic farming (NSW DPI, 2008; Pesticide Action Network UK, 2017).

Effect of wheat species

Since it was not possible to obtain information on the agronomic protocols used to produce the common and spelt wheat used to produce the flour samples included in the survey it is not possible to determine the exact reasons for the differences in pesticide residues between spelt and common wheat detected in the SBS reported here.

However, the finding that spelt wheat flour had only half the CCC and total CPP residue concentration compared with common wheat flour (Tables 4.14 and 4.15), could have been due to either (a) fewer CPPs having been applied to spelt crops and/or (b) the husk surrounding the Spelt grain having provided partial protection against the grain becoming contaminated. Spelt wheat is often described as a more stress resistant, robust species than modern common wheat cultivars (Rüegger and Winzeler, 1993). Also, protection by the husks is known to contribute to the higher levels of resistance in Spelt wheat against *Fusarium* head blight (Foroud and Eudes, 2009; Vučković *et al.*, 2013).

A significant difference in CPP residues between Spelt and common wheat was only detected when conventional flour samples were compared (Table 4.15.1), which suggests that switching from common to Spelt wheat consumption would results in a 50% reduction the total dietary CPP residue intake only for conventional but not organic food consumers .

Effect of flour type

There is increasing evidence for significant health benefits of switching from white to wholegrain cereal consumption (Jones and Engleson, 2010). However, conventional wholemeal flour contained more than 2-times higher CCC and CPP-residues than conventional white flour (Table 4.15.2), which is consistent with several previous studies, which reported higher concentrations of pesticides in bran compared with white flour (Weidenbörner *et al.*, 2000; Edwards *et al.*, 2011; Vidal *et al.*, 2013).

In contrast, CCC and total CPP residues concentrations in organic flour were not only substantially lower, but also similar in wholegrain and white flour (Table 4.15.2). This clearly demonstrates that organic wholegrain cereal consumption allows the nutritional benefits of wholegrain consumption (higher fibre, mineral and (poly)phenol/antioxidant intakes) to be achieved without a simultaneous increase in dietary exposure to pesticide residue.

Effect of country

The slightly higher CPP residues in conventional UK compared to German wheat suggest that the CPP spraying regimes are less intensive than those used in the UK (Table 4.15.3). However, in SBSs such as the one reported here it is not possible to obtain information on the agronomic and especially crop protection practices used to produce the wheat grain from which the flour was made. As a result, it is also not possible to identify potential reasons for the differences in CPP-residue levels between the two countries.

4.7.3 Conclusions

Results from this study suggest that switching to organic cereal consumption can substantially reduce dietary exposure to CPP residues and CPPs suspected or confirmed as endocrine disruptors. However, since residue levels were below the MRLs set for specific active ingredients/substances used in CPPs, this level of exposure is still considered safe by regulators.

Due to the uncertainty about the potential effects of exposure to mixtures of CPP residues and the lack of testing for epigenetic effects of exposure to endocrine disrupting CPP there is an urgent needs to investigate the potential physiological and health impact of reducing pesticide exposure via switching to organic food consumption. There is a particular need for controlled dietary intervention studies with animal models and humans, to provide a mechanistic understanding for associations between organic food consumption and positive health outcomes associated with organic food consumption reported in recent human cohort studies (Barański *et al.*, 2017).

4.8 Limitations

SBSs such as the one presented here aim to quantify (and estimate variation) phenolic phytochemical, mineral, mycotoxin and pesticide levels in the currently available flour brands available to consumers. However, it is virtually impossible to obtain information on specific farming system parameters known to affect levels of these parameters which include the (a) varieties of spelt and common wheat used, (b) location and pedo-climatic conditions and agronomic practices on cereal producing farms, (c) harvest parameters (e.g. climatic conditions, grain dry matter content), (d) post-harvest treatments used (e.g. grain drying and cleaning) and (e) quality assurance systems used to minimise mycotoxin contamination for the grain used to make the flour samples taken in supermarkets. It is also likely, that flour was made from cereal batches produced in a wide range of different regions and imported grains in both countries. Thus, it is impossible to provide definitive explanations for the differences in phenolic phytochemical, mineral, mycotoxin and pesticide concentrations reported here.

4.9 Conclusion

The flour survey based on all spelt and common wheat flour brands that could be found on the shelves of major UK and German retailer should reflect the main wheat varieties and flour products of the flour mills/supply chains in these countries. The findings suggested that switching to organic wholemeal flour allows for higher intakes of antioxidant/(poly)phenolics and mineral micro-nutrients which have been associated with potential health benefits of consuming wholegrain foods to be achieved without simultaneously increasing dietary exposure to pesticides. This is because the results showed that higher antioxidant concentrations and essential mineral micronutrients concentrations such as Mg, Fe, Zn, Cu were higher in wholegrain cereal at the same time that conventional wholemeal flour had more than 2-times higher total pesticide concentrations than conventional white flour. For the undesirable compounds mycotoxin, white and wholegrain flours contained similar concentrations of the mycotoxins DON, ZEA and OTA, and although T-2/HT-2 concentrations were significantly higher in wholegrain than white flour, T-2/HT-2 concentration in wholegrain flour were more than 10 times lower than the recommended maximum contamination levels set by the EU. The results therefore suggest that switching from white (refined) flours to wholegrain flours does not pose health risks associated with mycotoxin consumption.

Therefore, this survey suggested that the only negative nutritional trade-off associated with conventional wholegrain cereal consumption is the higher intake of pesticide residues, and that this trade-off can be avoided by switching to organic food consumption.

Chapter 5 Development of organic bread making minor cereal production systems for semi-arid regions in southern Europe; Spelt wheat (*Triticum Spelta; Triticum dicoccum*)

5.1 Introduction

It's widely known that spelt wheat is well adapted to cool and wet pedo-climatic conditions of higher altitudes and is therefore described as a 'robust' cereal (Rüegger and Winzeler, 1993). Spelt wheat has not, to our knowledge, been previously grown in semi-arid regions of the Mediterranean. As a result, there is no agronomic knowledge with respect to optimum sowing and harvest dates, tillage/mechanical weed control, fertility management, irrigation and crop protection protocols and the most suitable varieties for semi-arid regions of the Mediterranean. The most important yield and quality limiting factors in semi-arid crop production systems are irrigation, fertility management and the interactions between them (Garrido-Lestache *et al.*, 2004; Hussain *et al.*, 2010; Ma *et al.*, 2015) . Irrigation can increase grain yield as well as lead to a decrease on grain protein content mainly because of dilution effects (Guttieri *et al.*, 2005). Nitrogen, phosphorus, and potassium are the major nutrients which influence the productivity and quality of wheat in both winter rain-fed and irrigated production in the Mediterranean (Cabrera-Bosquet *et al.*, 2009).

Animal manures and organic waste-based composts are the main fertiliser inputs used in organic and other low-input cereal production systems. They contain low levels of water-soluble, readily-plant-available forms of N, P and K. As a result, the availability of N, P, and K added with organic fertilisers depends on microbial mineralisation of the organic matter in the soil. Microbial activity and associated mineralisation capacity in soil relies on optimum soil moisture and temperature conditions. As a result, the (a) total nutrient supply/availability to crops and (b) pattern of supply during the growing season and (c) crop yield and quality parameters in organic production (which uses organic fertilisers) would be expected to rely, to a much larger extent, on provision of optimum soil water conditions (for both mineralisation and direct water needs of crop plants) (Konopka *et al.*, 2012a). However, it is important to consider that NPK release patterns from organic fertilisers and the uptake of minerals by crop plants is affected by a combination of factors including soil type, irrigation system, temperature, soil water content (Magkos *et al.*, 2006).

Previous studies with common wheat (*T. aestivum*) demonstrated that macronutrient supply (especially N and P) and fertiliser type (organic vs mineral NPK) not only affects grain yield, but also the nutritional composition of cereals. High rates of applied N can increase grain yield (Garrido-Lestache *et al.*, 2004) and protein content from 7.8 to 11.3% (Campillo *et al.*, 1999) but might also decrease the total phenolic and antioxidant content (Konopka *et al.*, 2012b).

High mineral P-fertiliser inputs were linked to higher cadmium (Cd) concentrations in cereals/cereal products, even in regions (e.g. the European Union) where legislation is in place to ensure that mineral P-fertilisers with a low Cd content are used by farmers (Cooper et al. 2011; Baranski et al. 2014).

A recent study comparing the performance of traditional (grown until the 1960's) and modern common wheat varieties in organic production systems in the US also showed that mineral micronutrient concentrations (e.g. Se, Zn) were significantly lower in modern compared with traditional varieties (Murphy *et al.*, 2008)

The main **objectives** of this study where therefore to: (i) assess the effect of fertilisers with contrasting water-soluble N and P concentrations (sheep and chicken manure compost versus mineral NPK) on crop health and grain yield and nutritional quality parameters of spelt wheat varieties grown under semi-arid conditions; (ii) assess the effect of using supplementary irrigation in rain-fed winter spelt cereal crops on crop health and grain yield/yield stability and nutritional quality parameters; (iii) compare crop health, yield and quality parameters in (a) traditional "pure" spelt genotypes and (b) "modern" varieties based on *T. aestivum* x *T. spelta* crosses; (iv) identify interactions between contrasting spelt wheat varieties and agronomic parameters (irrigation and fertiliser regimes) with respect to crop health, yield, yield stability and grain quality parameters; (v) study the effect of contrasting climatic conditions (temperatures and especially rainfall is very variable between years in semi-arid regions of the Mediterranean) on spelt wheat performance.

5.2 Materials and Methods

5.2.1 *Field experimental design*

Spelt wheat was grown in an existing long-term field experiment at the Livadopa research station in Crete, Greece (Figure 5.1). The Livadopa trial has a factorial split-split-split plot design with 4 replicate blocks and (1) fertiliser type as main plot factor, (2) irrigation as the sub-plot factor and (3) variety choice as the sub-sub-plot factor (the design for two blocks are shown in figure 5.2). The three agronomic protocols compared were therefore: (1) fertiliser type and levels: composted chicken manure, composted sheep manure and mineral NPK applied at a total N-input level of 100kg N ha^{-1}; (2) irrigation (with or without irrigation) and (3) variety choice (the following four spelt varieties were compared):

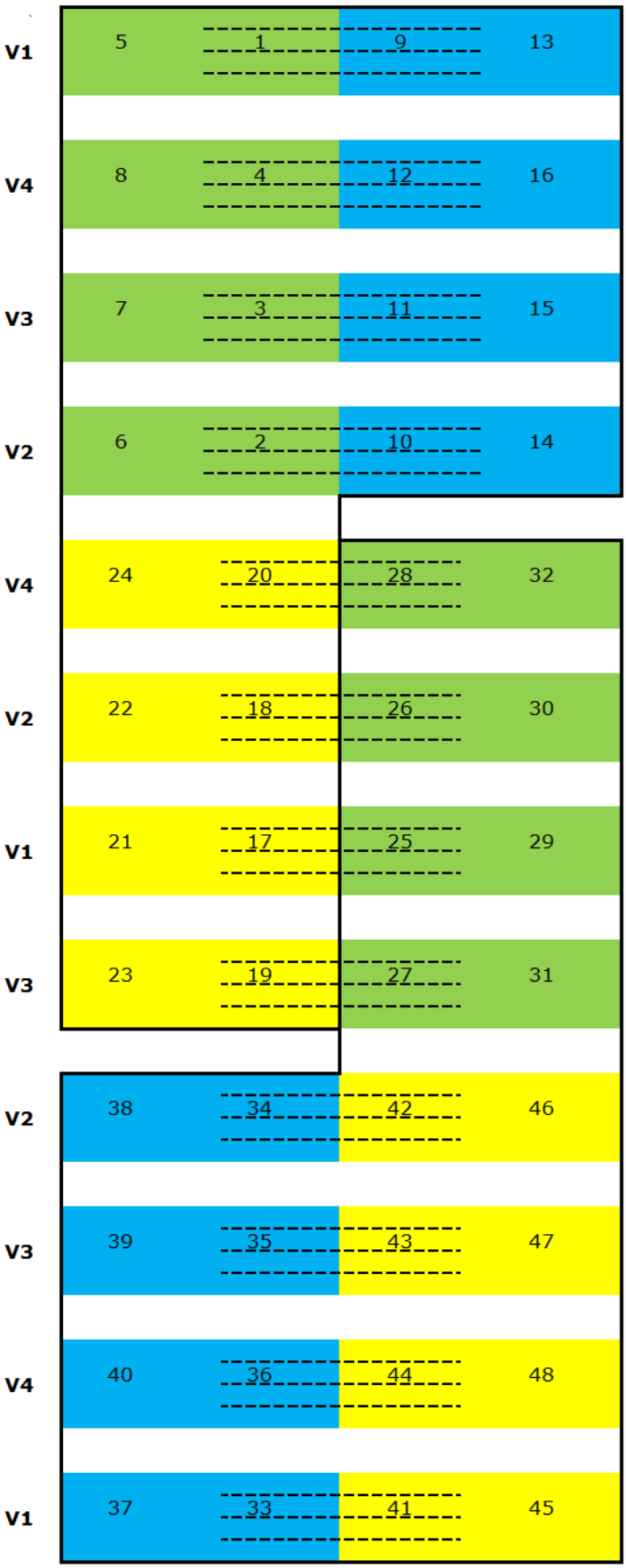
(i) Filderstolz (FIL), a short straw variety based on a cross between spelt and a high yielding German common wheat variety developed by Hohenheim University;

(ii) Oberkulmer (OBE), a long straw traditional Swiss variety marketed as a “true” or “pure” spelt wheat containing no common wheat genetics;

(iii) Rubiota (RUB), a long straw, traditional Czech spelt wheat variety; not thought to be based on crosses with *T. aestivum*;

(iv) Zürcher Oberländer Rotkorn (ZOR), a Sativa variety bred for organic production and selected under organic farming conditions by Peter Kunz.

Figure 5.1 Field experiment in Crete



Spelt Variety

1 RUBIOTA
2 FILDERSTOLZ
3 OBERKULMER
4 ZURCHER OBERLANDER ROTKORN

Fertility Treatments

1 = Sheep manure
2 = Chicken compost
3 = Mineral fertilizer

Irrigation regime

= With irrigation

= Without irrigation

Fertiliser input level

100 kg N ha-1

Area

Irrig Sub-Subplot	:	6	m^2
Fert subplot	:	12	m^2
Main fert plot	:	72	m^2
Total area	:	864	m^2

5	1	9	13
8	4	12	16
7	3	11	15
6	2	10	14
24	20	28	32
22	18	26	30
21	17	25	29
23	19	27	31
38	34	42	46
39	35	43	47
40	36	44	48
37	33	41	45
67	71	63	59
68	72	64	60
66	70	62	58
65	69	61	57
83	87	55	51
81	85	53	49
82	86	54	50
84	88	56	52
75	79	95	91
73	77	93	89
76	80	96	92
74	78	94	90

Figure 5.2 Experiment field design in Crete

5.2.2 *Field data collection*

Grain Yields were assessed at growth stage 85 soft dough (GS85) (AHDB, 2018) (just before the growth stage for harvest, and grains were harvested in slightly in advance of harvest growth stage because grain dropping resulting from too dry stems was a significant problem with the high temperatures in the field station and under extremely strong sun, and cause yield loss) by harvesting grain and straw from four 0.25m^2 quadrats from each plot. Ears were separated from straw and grains were separated and de-hulled by using a commercial cereal thresher. 200g grain samples were then milled into fine powder (<1mm) using a centrifuge mill (RETSCH ltd., Germany) and flour powder was stored in -80^{o}C until the determination of grain quality parameters as described in Chapter 3.

Lodging: three representative quadrants (50cm*50cm) were chosen in each plot for lodging assessment. Percentage and angle of lodging for each quadrant were recorded for calculation using the following equation, and expressed with the unit of %.

$$lodging\ score = lodging\ percentage\ \times lodging\ angle$$

Biomass and Harvest Index: Samples from three representative quadrants (50cm*50cm) were collected from each plot. Number of ears and stalks in each quadrant was counted for the biomass calculation. The weight of ears and stalks was used for harvest index calculation.

$$Harvest\ Index = \frac{Weight\ of\ ears}{Weight\ of\ stalks + Weight\ of\ ears} \times 100\%$$

Grain/Hull ratio: Ten representative ears from each plot were collected and weighed. Samples were then processed (using the thresher) to separate hull and grain. For grains in which the hull was still attached after threshing, hulls were removed by hand. Dehulled grains were weighed again. The Grain/Hull ratio was calculated as weight of grain/weight of hull.

Thousand grain weight (TGW): One thousand representative dehulled grains were counted and weighted to get the thousand grain weight (TGW) for each plot.

Leaf chlorophyll content (SPAD): ten representative leaves (2/3 of the distance from the leaf base to the apex) in each plot were assessed by chlorophyll meter SPAD and averaged at growth stage 39 flag leaf blade all visible (GS39), growth stage 50 first spikelet of ear just visible above flag leaf ligule (start of ear emergence) (GS50), growth stage 62 start of flowering (GS62).

Plant height (the shortest distance between the upper boundary of the main photosynthetic tissues on a plant and the ground level) of ten plants in each plot was measured using a ruler and averaged at grow stage 62 (GS62) start of flowering.

5.2.3 *Analysis of grain phenolic and flavonoid content, total antioxidant capacity and Macro and micronutrient content*

See Chapter 3, Methodology

Note that since only total phenolic content by Folin assay and total antioxidant capacity by TEAC and FRAP related antioxidants were analysed in the grain samples from this field trial, phenolic profiles by HPLC were not determined. Therefore, unlike the results presented in Chapter 4, in this Chapter, the estimated concentration of phenolic phytochemicals includes only total phenolic content and total flavonoid content.

5.2.4 *Statistical analysis*

Nonlinear mixed-effect models (Pinheiro and Bates, 2000) were used to analyse the data in a series of analysis to produce ANOVA *p*-values for main effects and all interactions using the nlme (non-linear mixed effects) package in R software (Pinheiro and Bates, 2000). A four-factor analysis (ANOVA) with year, fertiliser type, irrigation and variety choice as fixed effects was carried out. The hierarchical nature of the split-split plot designs was reflected in the random error structures that were specified as block/ year/ fertiliser type and irrigation type. The normality of the residuals of all models was tested using QQ-plots. Differences between the fertiliser types and the varieties as well as the interaction between factors were tested by using *''Tukey''* contrasts in the general linear hypothesis testing (glht) function of the multcomp package in R. A linear mixed effects model was used for the *''Tukey''* contrasts, containing a treatment main effect, with the random error term specified as described above.

5.3 Results

The field experiment was carried out in three years from 2015 to 2017. In 2015, there was only one variety (Rubiota) included and only field data (growth parameters) were collected. In 2016 and 2017, all four varieties were included and both field data and nutritional parameters assessed. In the main thesis, results for nutritional quality and yield assessment are reported and discussed (Tables 5.1 to 5.4). In order to further investigate the effect of contrasting climatic conditions (especially in the influence of high 2015 winter/spring precipitation) on spelt grain yield and protein content, a second analysis was performed including data from three years (2015, 2016 and 2017) but using only the variety (Rubiota) for which data from all 3 years was available. Since these data are less important for the main theme of the thesis, yield data are only presented in appendices 5.1 to 5.9.

5.3.1 *Effects of fertiliser type, irrigation, variety choice and harvest year on mineral, toxic metal, phenolic and flavonoid concentrations in spelt wheat grain*

Fertiliser type

Minerals and toxic metals: Significant main effects of fertiliser type were detected for N, P, S, Mg, Fe and Mo concentrations in grains (Tables 5.1 and 5.2). The effect of fertiliser types on grain composition were relatively small (<10% for most parameters for which significant differences were found). The use of mineral NPK fertilisers resulted in slightly but significantly higher N, S, Fe, and slightly, but significantly higher P, Mg, Mo concentrations were found in crops fertilised with sheep or chicken manure (Tables 5.1 to 5.2).

Concentration of phenolic phytochemicals and total antioxidant capacity: Only a significant main effect of fertiliser type on total phenolic was detected (Table 5.3). Mineral NPK fertiliser use resulted in significantly higher concentrations of total phenolics than both chicken and sheep manure.

Irrigation

Minerals and toxic metals: Significant main effects of irrigation were detected for N, S, Mg and Fe concentrations in grains (Tables 5.1 and 5.2). Concentrations of S and Fe were significantly higher (approx. 18% and 10% respectively) in non-irrigated plots, while concentrations of Mg and N were significantly higher (approx. 10% and 20% respectively) in irrigated crops. There was no significant main effect of irrigation on any of the other grain mineral and toxic metal levels assessed.

Concentration of phenolic phytochemicals and total antioxidant capacity: No significant main effects of irrigation on concentrations of phenolic and flavonoid and total antioxidant capacity were detected (Table 5.3).

Variety choice

Minerals: Variety choice was the experimental factor which had the greatest impact on grain composition. Significant main effects of variety were detected for all macro and micro-nutrients, but not Na, Al, Ni and Cd (Tables 5.1 and 5.2).The “modern” short straw variety Filberstolz (FIL; which is based on a cross between *T. aestivum* and *T. spelta*) had significantly lower N (13%), Mn (18%), Cu (79%), Fe (12%), Zn (23%), but significantly higher K (17%) than the other 3 varieties (Tables 5.1 and 5.2). FIL also had the lowest Cu concentrations, but the difference between ZOR and FIL was not significant (Table 5.2). The two traditional spelt varieties from Switzerland (Oberkulmer, OBE) and the Czech republic (Rubiota, RUB) had significantly higher N, S and Mg concentrations than the other 2 varieties (Table 5.1). Although significant, it should be pointed out, that the differences in grain P, K, Ca, Mg and Mo concentrations between varieties were relatively small (Tables 5.1 and 5.2).

Concentration of phenolic phytochemicals and total antioxidant capacity: Significant main effects of variety on total phenolic and flavonoids concentration and total antioxidant capacity by both FRAP and TEAC were detected (Table 5.3). The variety FIL had highest concentrations of total phenolics and flavonoids and total antioxidant capacity and the variety ZOR had lowest among those four varieties with significant differences (Table 5.3). FIL had approximately 25% higher phenolic acid, 40% higher flavonoid concentrations and 35% higher total antioxidant capacity than ZOR.

Harvest year

Minerals and toxic metals: Significant main effects of harvest year/growing season were detected for K, Ca, Mn, Na, Mo, and Al concentrations in grains (Tables 5.1 and 5.2). Concentrations of K, Ca, Na and Al were higher in 2016 (the year with exceptionally low rainfall), while concentration of Mn, Mo and total phenolic acids and flavonoids and total antioxidant capacity were significantly higher in 2017 (the higher rainfall year). There was no significant effect of growing year on N, P, S, Mg, Cu, Fe, Zn, Ni and Cd concentrations.

Concentration of phenolic phytochemicals and total antioxidant capacity: Significant main effects of harvest year were detected for total phenolics, flavonoids in grain and total antioxidant capacity of grain. Concentrations were lower in 2016 (the year with exceptionally low rainfall) than 2017 (the higher rainfall year) (Table 5.3).

Interactions

Significant interactions between harvest year and fertiliser type were detected for spelt grain N and Mo concentrations and total antioxidant capacity (TEAC) (Tables 5.1 to 5.3). Grain N concentrations were significantly higher when mineral N fertiliser was used in both harvest

years, but the relative differences between fertiliser treatments were greater in 2007 (the wetter year) (Figure 5.1.1). Grain Mo concentrations were lowest in mineral N and highest in chicken manure fertilised crops, but the relative differences between fertiliser treatments were greater in 2007 (the wetter year) (Figure 5.2.2). Antioxidant capacity was similar with all 3 fertiliser types in 2016 (the dryer year), while in 2017 antioxidant capacity in grain from sheep manure fertilised plots was slightly, but significantly higher than in mineral N and chicken manure fertilised plots (Figure 5.3.1).

Significant interactions between harvest year and irrigation were identified only for grain Mo concentrations. In 2006 (the dryer year) Mo-concentrations were significantly higher in irrigated plots, while there was no significant effect of irrigation in 2007 (Figure 5.2.1)

Interactions between year and variety were identified for grain magnesium (Mg), manganese (Mn) and total phenolic acid concentrations and total antioxidant capacity (in both the FRAP and TEAC assay) (Tables 5.1, 5.2 and 5.3). Grain K-concentration: The differences between varieties had nearly identical trends in both years, and the nature of this interaction was therefore not possible to determine (Figure 5.1.2). Grain Mg-concentrations: OBE and RUB had significantly higher grain Mg concentrations than the FIL and ZOR. However, the relative differences between varieties were larger in 2016 (the dryer year) than 2017 (Figure 5.1.3). Grain Mn-concentrations: In both years FIL had significantly lower Mn-concentrations than the other 3 varieties. However, in 2016 (the dryer year) ZOR had the highest Mn-concentrations, while in 2017 the highest Mn-concentrations were found in OBE (Figure 5.2.3). Total phenolic acid concentrations: In both years FIL had the highest and ZOR had the lowest phenolic acid concentrations, but in 2016 the difference between FIL and OBE was not significant (Figure 5.3.3). Antioxidant capacity (FRAP and TEAC): Results obtained with both antioxidant assays showed very similar trends. In both years FIL had significantly higher activity than ZOR, with the other two varieties showing intermediate levels of antioxidant capacity. However, in the relative differences between varieties were greater in 2017, when antioxidant capacity was approximately 2 time higher than in 2016 in all varieties (Figure 5.3.4 and 5.3.5).

Interactions between variety and irrigation were detected for grain copper (Cu) and total flavonoid concentrations (Tables 5.2 and 5.3).Grain Cu-concentrations: In irrigated plots RUB had the highest Cu concentrations, while in winter rain-fed non-irrigated plots OBE had the highest Cu concentrations. Also, FIL had the lowest Cu-concentrations in non-irrigated and ZOR in irrigated plots (Figure 5.2.4). Flavonoid concentrations: In both irrigated and non-irrigated plots flavonoid concentrations were highest in FIL and lowest in ZOR, with OBE and RUB having intermediate concentrations. However the relative differences between varieties were greater in irrigated plots. (Figure 5.3.2).

Interactions between variety and fertiliser type were detected for grain manganese (Mn) and molybdenum (Mo) concentrations (Tables 5.2 and 5.3). Grain Mn-concentrations: With all 3 fertiliser types Mn concentrations in OBE and ZOR were higher than in FIL and RUB, but the relative differences between varieties were smaller with sheep manure as fertiliser when compared to chicken manure and mineral NPK (Figure 5.2.5). Grain Mo-concentrations: There was no significant difference in Mo concentrations between varieties when mineral NPK was used. In contrast, when chicken and sheep manure were used significant differences between varieties were detected with FIL showing the highest and OBE the lowest concentrations (Figure 5.2.6).

5.3.2 *Effects of fertiliser type, irrigation, variety choice and harvest year on spelt wheat (variety Rubiota) grain yield parameters*

Fertiliser type

There were significant main effects of fertiliser type (chicken manure vs mineral NPK vs composted sheep manure) on all other crop performance/growth parameters (grain protein, harvest index, plant height, lodging, ears per m^2, grain hull ratio, TGW, and leaf chlorophyll levels,) except plant height and tiller numbers (Table 5.4). Grain yield, harvest index, ear number, grain hull ratio and TGW were lower in mineral-NPK than chicken or sheep manure fertilised crops. In contrast, plant height, % lodging and leaf chlorophyll levels were higher in mineral-NPK than chicken or sheep manure fertilised crops (Table 5.4).

Irrigation

There were significant main effects of irrigation on grain yield and all other crop performance/growth parameters (harvest index, plant height, lodging, tillers and ears per m^2, grain/hull ratio and TGW) but not chlorophyll concentration (Table 5.4). Grain yield and all other growth parameters (except for chlorophyll concentrations) were higher in irrigated plots. In contrast, leaf chlorophyll (SPAD) concentrations were higher in non-irrigated plots (Tables 5.4).

Variety choice

Significant main effects of variety were detected for grain yield and all other performance/growth parameters except tiller number and grain/hull ratio (Table 5.4). The highest grain yields were obtained with the Sativa variety Züricher Oberländer Rotkorn (ZOR) with yields being significantly higher than those of the varieties Filderstolz (FIL; the 2nd highest yielding variety, which is based on a *T. aestivum* x *T. spelta*), Oberkulmer (a traditional, long-straw Swiss variety) and the lowest yielding variety Rubiota (RUB; a traditional, long-straw Czech variety) (Table 5.4).

The tallest varieties were RUB and ZOR which had significantly longer straw than OBE and FIL, which had a significantly shorter straw that the other 3 varieties (Table 5.4). Lodging severity has highest in the two long straw varieties (RUB and ZOR) and lowest in the variety with the shortest straw (FIL) (Table 5.4). The variety FIL produced a significantly higher number of ears than the other 3 varieties, but the difference with ZOR was not significant (Table 5.4). The varieties ZOR and OBE produced a significantly higher TGW than the other two varieties (FIL and RUB) and the TGW of FIL was significantly lower than that of the other 3 varieties (Table 5.4). The two traditional varieties from the Czech Republic (RUB) and Switzerland (CH) had significantly higher grain protein concentrations than the more modern

varieties (FIL and ZOR) and grain protein levels of FIL were significantly lower than those reported for the other 3 varieties (Table 5.4).

Harvest year/season

There were significant main effects of growing year on grain yield and most other crop performance/ growth parameters (harvest index, plant height, lodging, grain/hull ratio and TGW) expect for tillers per m2 (Table 5.4). All other performance/growth parameters (except lodging) were higher in 2017 (the year with higher rainfall) compared with 2016 (Table 5.4). However, for tillers per m2 and chlorophyll levels (SPAD), which was used as an indication of N-supply), no significant main effect of growing year could be detected (Table 5.4).

Interactions

Two-way interactions between year and fertiliser type were detected for plant height, tiller, ear numbers and grain/hull ratio (Table 5.4). Mineral NPK use resulted in significantly longer straw than chicken and sheep manure in 2017 (the wetter year), but not in 2016 (Figure 5.4.1). Mineral NPK use resulted in significantly lower tiller numbers than chicken and sheep manure in 2016 (the dryer year), but significantly higher tiller numbers in 2017 (Figure 5.4.2). Mineral NPK use resulted in significantly lower ears number in 2016 by not in 2017 (Figure 5.4.3). Mineral NPK use resulted in a significantly lower grain/hull ratio than chicken and sheep manure in 2016 (the dryer year), while there was no significant effect of fertiliser type in 2017 (Figure 5.4.4).

Significant interactions between year and irrigation were detected for the number of tillers and the grain/hull ratio (Table 5.4). There was no significant difference in tiller numbers between year in irrigated crops; while in non-irrigated plots tiller numbers were significantly higher in 2017 (the wetter year) than 2016 (Figure 5.4.5). There was no significant difference in the grain/hull ratio between irrigated and non-irrigated plots in 2007 (the wetter year), while the grain/hull ratio was significantly higher in irrigated plots in 2016 (the dryer year) (Figure 5.4.6).

Significant interactions between year and variety were detected for grain yield, harvest index, lodging severity and ear number (Tables 5.4). There was no difference in yield between varieties in 2016 (the drier year), while ZOR produced significantly higher yields than the other 3 varieties, and the lowest yield were recorded for the two traditional varieties (OBE and RUB) (Figure 5.4.7). The harvest index was highest for FIL and lowest for ZOR in 2016 (the drier year), but highest for ZOR and lowest for OBE in 2017 (Figure 5.4.8). In 2016 lodging severity in FIL plots was significantly lower than the other 3 varieties, whereas in 2017 lodging severity in FIL, OBE and ZOR plots was similar but lower than in RUB plots (Figure 5.4.9). In 2016 (the drier year) ZOR produced a lower number of ears than the 3 other varieties, but the difference

with RUB was not significant, whereas in 2017 ZOR produced a significantly higher number of ears than the 3 other varieties (Figure 5.4.10).

A significant interaction between irrigation and variety choice was only detected for grain yield (Table 5.4). With irrigation the variety ZOR produced significantly higher yields than the other 3 varieties, while RUB produced significantly lower grain yields than the other 3 varieties. In contrast, in winter rain-fed, non-irrigated plots there was no significant difference in grain yields between varieties (Figure 5.4.11).

Significant 3-way interactions between year, irrigation and fertiliser type were detected for grain yield and harvest index (Tables 5.4). In 2016 (the drier year), mineral fertiliser use resulted in lower grain yields than both chicken and sheep manure when irrigation was applied, while there was no significant difference in grain yield between fertiliser types in non-irrigated plots (Table 5.4.1). In contrast, in 2017 (the wetter year) mineral fertiliser use resulted in lower grain yields than both chicken and sheep manure in non-irrigated plots, while there was no significant difference in grain yield between fertiliser types in irrigated plots (Table 5.4.1). In 2016 (the drier year) all 3 fertiliser types were in higher harvest index when irrigation was used. In contrast, in 2017 (the wetter year) irrigation resulted in higher harvest index only when chicken manure or mineral NPK was used as fertiliser, while irrigation had no significant effect in plots fertilised with sheep manure (Table 5.4.1).

Table 5.1 Effects of Harvest year, irrigation, fertiliser type and variety choice on spelt wheat grain protein and macro-nutrient content

	Crude Protein	**mineral macronutrients**					
		Grain Na	**Grain P**	**Grain K**	**Grain S**	**Grain Ca**	**Grain Mg**
Means ±SE	%	mg kg DW	mg g DW	mg g DW	mg g DW	mg g DW	mg g DW
Year (n=96)							
2016	12.5 ±0.19	77.9 ±3.28	3.7 ±0.04	3.8 ±0.04	1.5 ±0.02	0.5 ±0.02	1.0 ±0.01
2017	13.0 ±0.33	44.6 ±1.26	3.8 ±0.04	3.5 ±0.04	1.5 ±0.02	0.4 ±0.01	1.1 ±0.01
Irrigation (n=96)							
With	11.7 ±0.18	58.3 ±2.92	3.8 ±0.04	3.6 ±0.04	1.4 ±0.02	0.4 ±0.02	1.1 ±0.01
Without	13.9 ±0.30	63.9 ±3.07	3.7 ±0.04	3.7 ±0.05	1.6 ±0.02	0.5 ±0.01	1.0 ±0.01
Fertiliser type (n=64)							
CHI*	12.0 ±0.24 **b**	65.3 ±4.27	3.8 ±0.04	3.7 ±0.05	1.4 ±0.02 **b**	0.5 ±0.02	1.1 ±0.01 **b**
MIN*	14.2 ±0.42 **a**	60.8 ±3.21	3.6 ±0.05	3.6 ±0.05	1.6 ±0.02 **a**	0.5 ±0.01	1.0 ±0.01 **a**
SHE*	12.1 ±0.23 **b**	57.3 ±3.44	3.7 ±0.04	3.6 ±0.06	1.4 ±0.02 b	0.4 ±0.01	1.1 ±0.01 **b**
Variety (n=48)							
FIL	11.4 ±0.25 **c**	62.1 ±4.52	3.8 ±0.04 **a**	4.1 ±0.06 **a**	1.4 ±0.02 **b**	0.5 ±0.01 **a**	1.0 ±0.01 **b**
OBE	13.4 ±0.26 **a**	61.3 ±4.28	3.9 ±0.05 **a**	3.6 ±0.04 **b**	1.6 ±0.02 **a**	0.5 ±0.01 **a**	1.1 ±0.01 **a**
RUB	13.7 ±0.29 **a**	58.9 ±4.20	3.9 ±0.05 **a**	3.6 ±0.05 **b**	1.6 ±0.02 **a**	0.5 ±0.03 **a**	1.1 ±0.02 **a**
ZOR	12.5 ±0.56 **b**	62.1 ±4.07	3.4 ±0.03 **b**	3.2 ±0.03 **c**	1.4 ±0.02 **b**	0.4 ±0.01 **b**	1.0 ±0.01 **c**
ANOVA							
Main effects							
Year (YR)	ns	**0.0322**	ns	**0.0358**	0.0775	**0.0289**	ns
Irrigation (IR)	**0.0010**	ns	ns	0.0667	**0.0003**	ns	**0.0335**
Fertiliser type (FT)	**<.0001**	ns	**0.0061**	ns	**<.0001**	ns	**0.0492**
Variety (SV)	**<.0001**	ns	**<.0001**	**<.0001**	**<.0001**	**0.0019**	**<.0001**
Interactions#							
YR x IR	ns	ns	ns	ns	ns	ns	ns
YR x FT	**0.0366** [1]	ns	ns	ns	ns	ns	ns
IR x FT	ns	ns	ns	ns	ns	ns	ns
YR x SV	ns	ns	0.0872	**0.0066** [2]	ns	ns	**0.0233** [3]
IR x SV	ns	ns	ns	ns	ns	ns	ns
FT x SV	0.0730	ns	ns	ns	ns	ns	ns

Means that are followed by the same letter within each column are not significant different (general linear hypothesis test $p<0.05$);
*CHI: chicken manure; *MIN: mineral fertiliser; *SHE: sheep manure;
1See figures 5.7.1 for interaction means ±SE; 2See figures 5.7.2 for interaction means ±SE; 3See figures 5.7.3 for interaction means ±SE
Interactions without significant differences were removed.

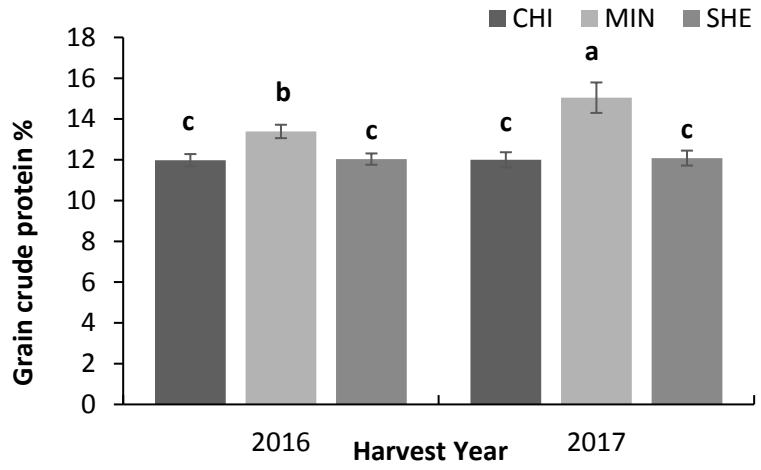


Figure 5.1.1 Effects of interaction between harvest year and fertiliser type on spelt wheat grain crude protein

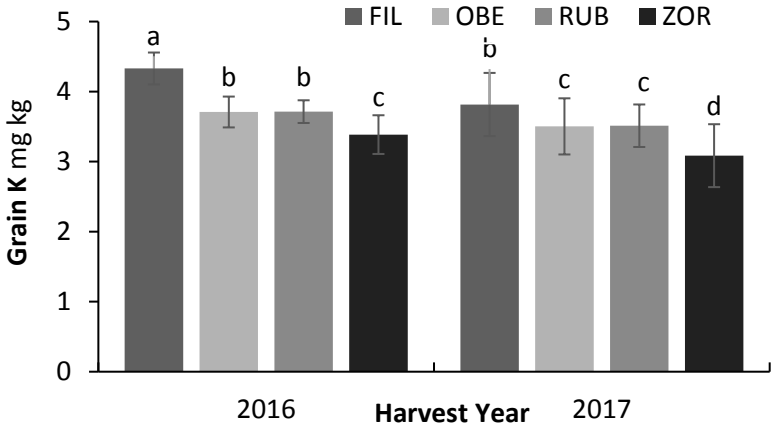


Figure 5.1.2 Effects of interaction between harvest year and variety choice on spelt wheat grain K content

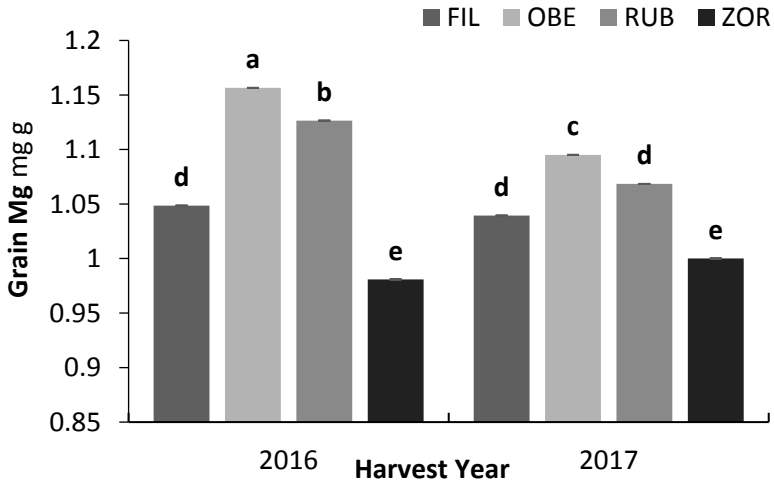


Figure 5.1.3 Effects of interaction between harvest year and variety choice on spelt wheat grain Mg content

Table 5.2 Effects of Harvest year, irrigation, fertiliser type and variety choice on spelt wheat grain micro-nutrient content and undesirable and toxic metals.

	mineral micronutrients					**undesirable and toxic metals**		
	Grain Fe	**Grain Mn**	**Grain Zn**	**Grain Cu**	**Grain Mo**	**Grain Al**	**Grain Ni**	**Grain Cd**
Means ±SE	mg kg DW	mg kg DW	mg kg DW	mg kg DW	mg kg DW	mg kg DW	mg kg DW	µg kg DW
Year (n=96)								
2016	36.8 ±0.60	26.4 ±0.38	36.9 ±0.72	7.9 ±0.17	0.7 ±0.02	9.8 ±0.58	0.8 ±0.07	99.9 ±18.29
2017	36.6 ±0.82	29.4 ±0.36	35.5 ±0.61	8.6 ±0.21	0.8 ±0.03	5.1 ±0.16	0.7 ±0.05	44.8 ± 3.58
Irrigation (n=96)								
With	34.7 ±0.66	28.1 ±0.38	36.2 ±0.63	8.1 ±0.18	0.8 ±0.02	7.5 ±0.62	0.8 ±0.08	55.8 ± 4.23
Without	38.7 ±0.72	27.7 ±0.42	36.2 ±0.70	8.4 ±0.21	0.7 ±0.03	7.4 ±0.3	0.7 ±0.05	89.4 ±18.52
Fertility type (n=64)								
CHI*	35.3 ±0.87 **b**	28.6 ±0.48	36.1 ±0.91	8.0 ±0.23	0.9 ±0.03 **a**	8.2 ±0.88	0.8 ±0.08	85.4 ± 25.75
MIN*	38.4 ±0.86 **a**	27.7 ±0.52	36.4 ±0.80	8.4 ±0.23	0.5 ±0.02 **c**	7.2 ±0.46	0.7 ±0.05	54.6 ± 4.63
SHE*	36.4 ±0.87 **b**	27.5 ±0.47	36.0 ±0.74	8.5 ±0.26	0.8 ±0.02 **b**	7.0 ±0.29	0.9 ±0.10	79.3 ± 13.46
Variety (n=48)								
FIL	33.2 ±1.03 **c**	24.0 ±0.42 **c**	29.5 ±0.50 **d**	7.9 ±0.25 **c**	0.9 ±0.04 **b**	6.7 ±0.42	0.7 ±0.10	56.8 ± 5.66
OBE	38.9 ±1.16 **a**	29.9 ±0.48 **a**	41.2 ±0.66 **a**	8.7 ±0.30 **a**	0.7 ±0.02 **a**	7.6 ±0.52	0.8 ±0.10	69.1 ± 14.00
RUB	36.8 ±0.90 **b**	27.6 ±0.52 **b**	38.4 ±0.96 **b**	8.5 ±0.26 **ab**	0.7 ±0.03 **ac**	8.3 ±1.15	0.8 ±0.08	61.7 ± 6.04
ZOR	37.9 ±0.78 **ab**	30.3 ±0.31 **a**	35.8 ±0.64 **c**	8.0 ±0.28 **bc**	0.8 ±0.04 **c**	7.2 ±0.40	0.7 ±0.07	103.4 ± 34.53
ANOVA results (p-values)								
Main effects								
Year (YR)	ns	**0.0322**	ns	0.1335	**0.0482**	**0.0040**	ns	0.0736
Irrigation (IR)	**0.0019**	ns	ns	ns	ns	ns	ns	ns
Fertility type (FT)	**0.0175**	0.068	ns	ns	**<.0001**	ns	ns	ns
Variety (SV)	**<.0001**	**<.0001**	**<.0001**	**0.0071**	**<.0001**	ns	ns	ns
Interactions#								
YR x IR	ns	ns	ns	ns	**0.0243** [1]	ns	ns	ns
YR x FT	ns	ns	ns	ns	**<.0001**[2]	ns	ns	ns
IR x FT	ns	ns	ns	ns	ns	ns	ns	ns
YR x SV	ns	**0.0003** [3]	ns	ns	ns	ns	ns	ns
IR x SV	ns	ns	ns	**0.0091** [4]	ns	ns	ns	ns
FT x SV	ns	**0.0387** [5]	ns	ns	**0.0041** [6]	ns	ns	ns

Means that are followed by the same letter within each column are not significant different (general linear hypothesis test $p<0.05$);
*CHI: chicken manure; *MIN: mineral fertiliser; *SHE: sheep manure; # Interactions without significant differences were removed.
1See figure 5.2.1 for interaction means ±SE; 2See figure 5.2.2 for interaction means ±SE; 3See figure 5.2.3 for interaction means ±SE;
4See figure 5.2.4 for interaction means ±SE; 5See figure 5.2.5 for interaction means ±SE; 6See figure 5.2.6 for interaction means ±SE;

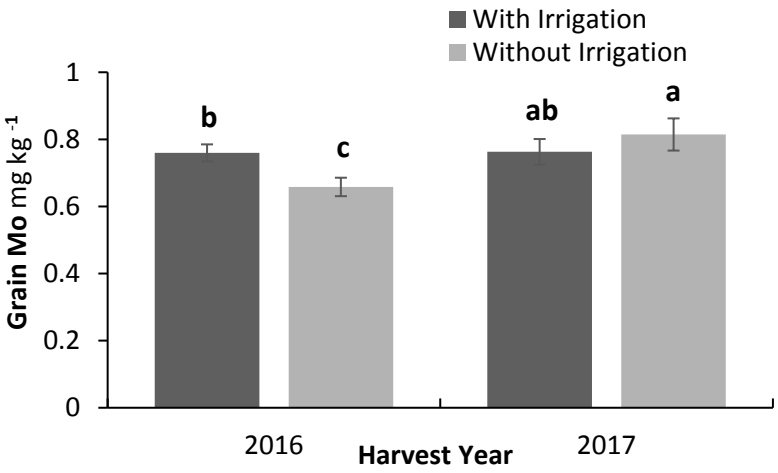


Figure 5.2.1 Effects of interaction between harvest year and irrigation on spelt wheat plant grain Mo content

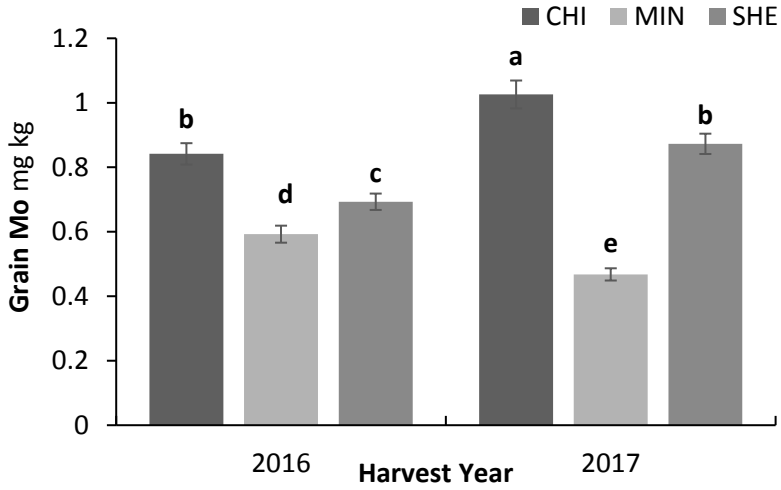


Figure 5.2.2 Effects of interaction between harvest year and fertility type on spelt wheat grain Mo content

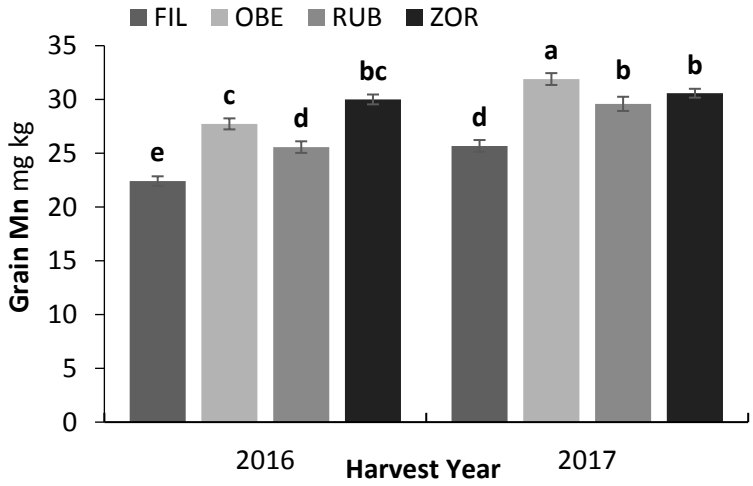


Figure 5.2.3 Effects of interaction between harvest year and variety choice on spelt wheat grain Mn content

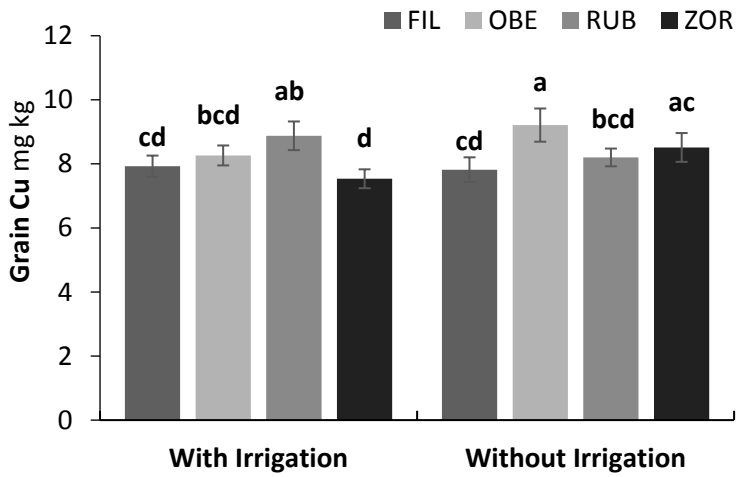


Figure 5.2.4 Effects of interaction between irrigation and variety choice on spelt wheat grain Cu content

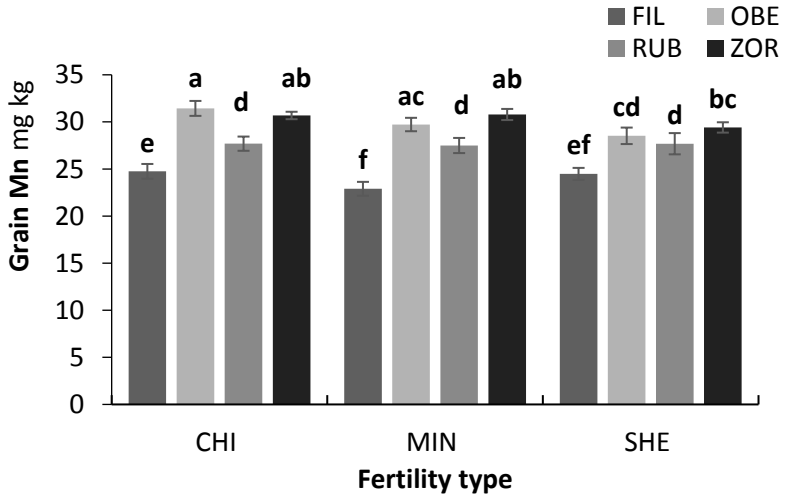


Figure 5.2.5 Effects of interaction between fertiliser type and variety choice on spelt wheat grain Mn content

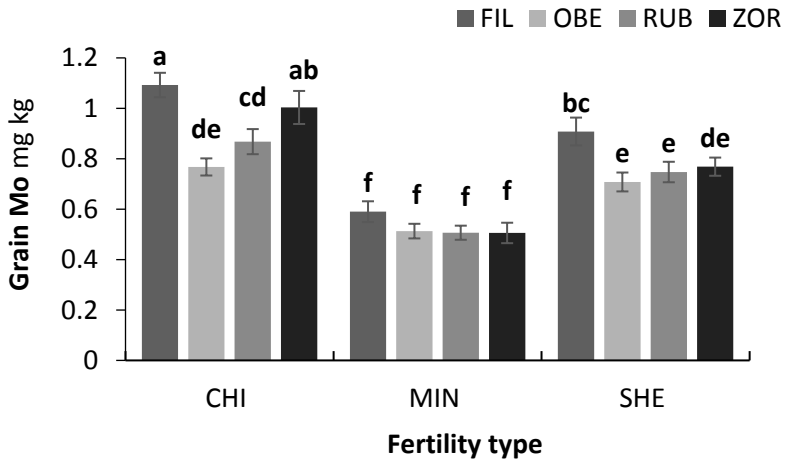


Figure 5.2.6 Effects of interaction between fertiliser type and variety choice on spelt wheat grain Mo content

Table 5.3 Effects of Harvest year, irrigation, fertiliser type and variety choice on total phenolic content, total flavonoid content and total antioxidant capacity (FRAP and ABTS) of spelt wheat grain.

	Total phenolic content	**Total favonoid content**	**Total Antioxidant capacity**	
			FRAP	**TEAC**
Means ±SE	umol GAE g^{-1}	umol Catechin g^{-1}	umol FeSO4.7H2O g^{-1}	umol Trolox g^{-1}
Year (n=96)				
2016	10.4 ±0.13	1.12 ±0.03	6.4 ±0.11	6.1 ±0.09
2017	11.6 ±0.16	1.39 ±0.03	13.5 ±0.28	13.3 ±0.23
Irrigation (n=96)				
With	10.9 ±0.16	1.27 ±0.04	10.2 ±0.44	9.9 ±0.42
Without	11.0 ±0.16	1.24 ±0.03	9.8 ±0.40	9.6 ±0.40
Fertility type (n=64)				
CHI*	10.7 ±0.18 **b**	1.23 ±0.043	10.1 ±0.51	9.6 ±0.48
MIN*	11.4 ±0.17 **a**	1.30 ±0.039	9.8 ±0.52	9.7 ±0.49
SHE*	11.0 ±0.21 **b**	1.23 ±0.042	10.2 ±0.53	10.0 ±0.54
Variety (n=48)				
FIL	12.1 ±0.22 **a**	1.45 ±0.04 **a**	12.0 ±0.70 **a**	11.5 ±0.67 **a**
OBE	11.4 ±0.15 **b**	1.26 ±0.04 **b**	9.8 ±0.53 **b**	9.5 ±0.53 **b**
RUB	10.9 ±0.16 **c**	1.24 ±0.04 **b**	9.5 ±0.54 **b**	9.4 ±0.54 **b**
ZOR	9.5 ±0.15 **d**	1.06 ±0.05 **c**	8.7 ±0.49 **c**	8.6 ±0.48 **c**
ANOVA results (p-values)				
Main effects				
Year (YR)	**0.0093**	**0.0264**	**0.0011**	**0.0001**
Irrigation (IR)	NS	NS	NS	NS
Fertility type (FT)	**0.0075**	NS	NS	NS
Variety (SV)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Interactions #				
YR x IR	NS	NS	NS	NS
YR x FT	NS	NS	NS	**0.0244** [1]
I R x FT	NS	NS	NS	NS
YR x SV	**0.0141** [3]	NS	**<0.0001** [4]	**<0.0001** [5]
IR x SV	NS	**0.0315** [2]	NS	NS
FT x SV	0.0980	NS	NS	NS

*CHI: chicken manure; *MIN: mineral fertiliser; *SHE: sheep manure; Means that are followed by the same letter within each column are not significant different (general linear hypothesis test $p<0.05$); # Interactions without significant differences were removed.

[1]See figures 5.3.1 for interaction means ±SE; [2]See figures 5.3.2 for interaction means ±SE; [3]See figures 5.3.3 for interaction means ±SE; [4]See figures 5.3.4 for interaction means ±SE; [5]See figures 5.3.5 for interaction means ±SE;

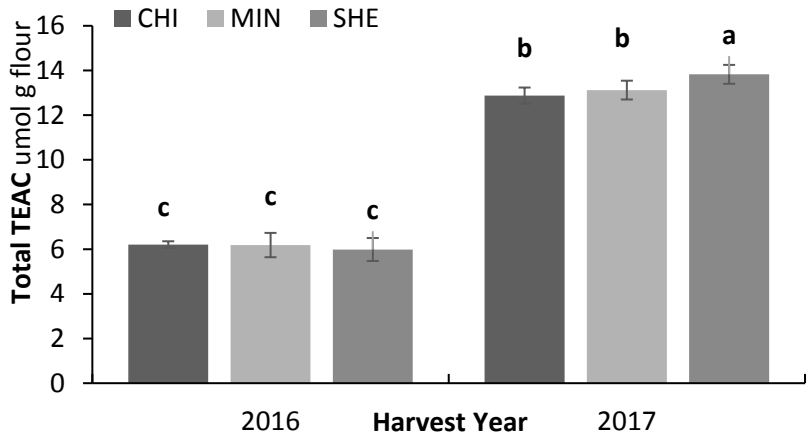


Figure 5.3.1 Effects of interaction between harvest year and fertiliser type on spelt wheat grain total antioxidant capacity (TEAC assay)

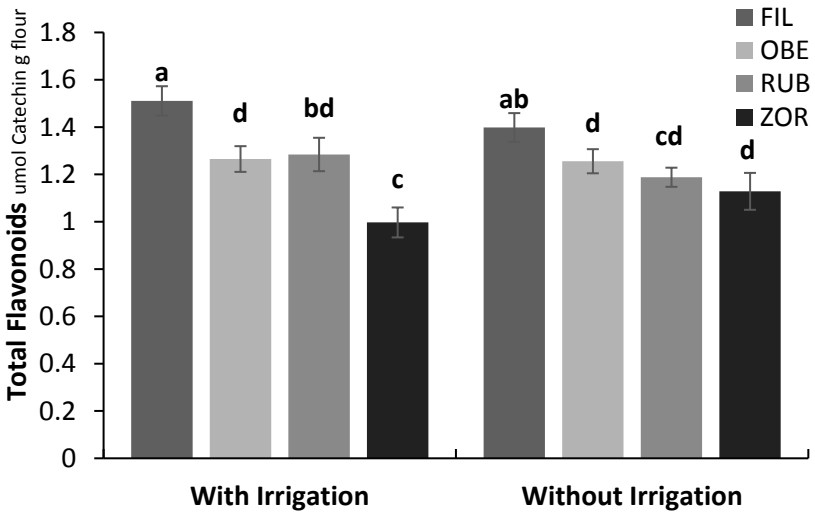


Figure 5.3.2 Effects of interaction between irrigation and variety choice on spelt wheat grain total flavonoids

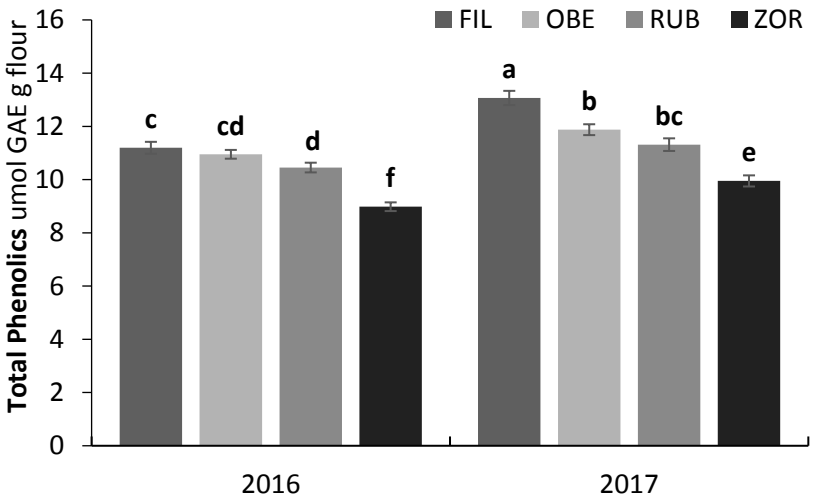


Figure 5.3.3 Effects of interaction between harvest year and variety choice on spelt wheat grain total phenolic compounds

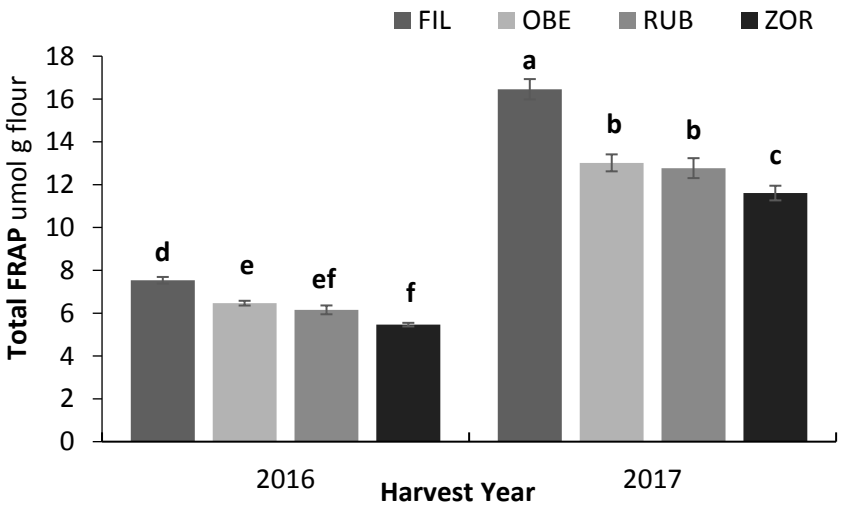


Figure 5.3.4 Effects of interaction between harvest year and variety choice on spelt wheat grain total antioxidant capacity (FRAP assay).

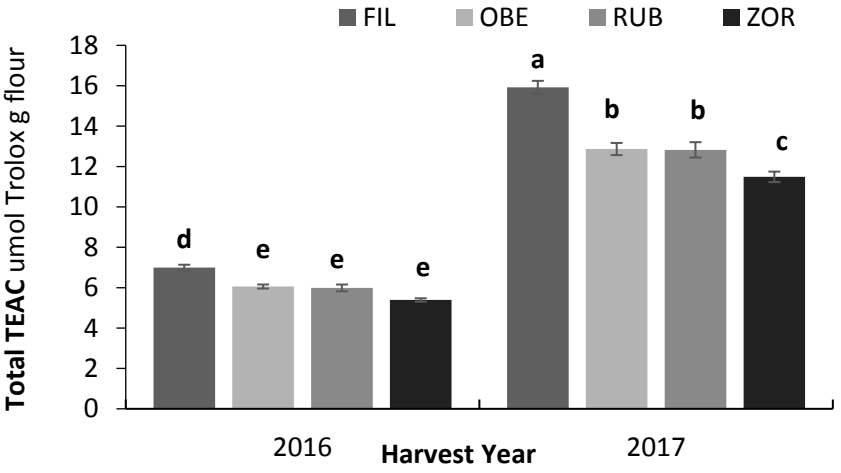


Figure 5.3.5 Effects of interaction between harvest year and variety choice on spelt wheat grain total antioxidant capacity (TEAC assay).

Table 5.4 Effects of Harvest year, irrigation, fertiliser type and variety choice on spelt wheat grain yield, harvest index, plant height and stem lodging, on spelt wheat yield components, SPAD as an estimation of leaf chlorophyll content

	Grain Yield	**Harvest Index**	**Plant height GS62**	**Lodging**	**Tillers/m²**	**Ears/m²**	**Grain/hull**	**TGW**	**SPAD GS39**	**SPAD GS50**	**SPAD GS62**
Means ±SE	t ha^{-1}	%	cm	%			%	g			
Year (n=96)											
2016	1.1 ±0.1	11 ±1	84 ±2	9±1	317 ±9	189 ±8	56 ±1	36.2 ±0.37	-	40.1 ±0.6	-
2017	2.7 ±0.2	23 ±1	107 ±2	4±1	335 ±7	284 ±8	67 ±0.4	40.2 ±0.54	41.4 ±0.5	45.2 ±0.4	45.3 ±0.4
Irrigation (n=96)											
With	2.7 ±0.2	20 ±1	108 ±2	9±1	363 ±7	280 ±9	65 ±1	40.2 ±0.46	39.6 ±0.6	41.6 ±0.6	44.3 ±0.6
Without	1.1 ±0.1	14 ±1	84 ±2	5±1	289 ±7	193 ±8	58 ±1	36.1 ±0.47	43.2 ±0.7	43.7 ±0.5	46.3 ±0.7
Fertility type (n=64)											
CHI	2.0 ±0.2 **a**	19 ±1 **a**	94 ±3	5±1 **b**	330 ± 9	249 ±11 **a**	62 ±1 **a**	38.8 ±0.60 **a**	40.2 ±0.7 **b**	41.9 ±0.5 **b**	43.8 ±0.7 **b**
MIN	1.7 ±0.2 **b**	13 ±1 **b**	98 ±3	10±1 **a**	328 ±11	217 ±14 **b**	58 ±2 **b**	35.9 ±0.58 **b**	45.2 ±0.7 **a**	44.9 ±0.7 **a**	48.4 ±0.8 **a**
SHE	2.0 ±0.2 **a**	20 ±1 **a**	94 ±2	5±1 **b**	321 ± 9	244 ± 9 **a**	64 ±1 **a**	39.8 ±0.58 **a**	38.9 ±0.6 **b**	41.2 ±0.8 **b**	43.8 ±0.5 **b**
Variety (n=48)											
FIL	2.0 ±0.2 **b**	19 ±1 **a**	80 ±3 **c**	3 ±1 **c**	320 ±11	258 ±11 **a**	60 ±1	36.1 ±0.74 **c**	44.0 ±1.0 **a**	45.0 ±0.8 **a**	47.0 ±0.8 **a**
OBE	1.7 ±0.2 **bc**	15 ±1 **c**	94 ±3 **b**	7 ±2 **ab**	322 ±13	229 ±14 **b**	60 ±2	39.8 ±0.79 **a**	40.5 ±0.9 **bc**	42.3 ±0.7 **b**	45.3 ±0.7 **bc**
RUB	1.5 ±0.2 **c**	16 ±1 **bc**	105 ±3 **a**	10 ±2 **a**	320 ±12	218 ±12 **b**	63 ±1	37.3 ±0.57 **b**	39.8 ±0.9 **c**	40.6 ±0.7 **c**	43.6 ±0.8 **c**
ZOR	2.4 ±0.3 **a**	17 ±2 **ab**	103 ±3 **a**	6 ±1 **b**	343 ±10	241 ±17 **ab**	63 ±2	39.5 ±0.61 **a**	41.6 ±0.8 **b**	42.8 ±0.9 **b**	45.5 ±1.0 **ab**
ANOVA result p-value											
Main effects											
Year (YR)	**0.0017**	**0.0012**	**0.0019**	**0.0283**	NS	**0.0033**	**0.0019**	**0.0082**	-	0.0848	-
Irrigation (IR)	**<0.0001**	**0.0005**	**<0.0001**	**0.0244**	**0.0004**	**0.0002**	**0.0011**	**0.0006**	**0.0352**	**0.0124**	NS
Fertility type (FT)	**0.0074**	**<0.0001**	ns	**0.0019**	NS	**0.0167**	**0.0005**	**0.0001**	**<0.0001**	**<0.0001**	**0.0016**
Variety (SV)	**<0.0001**	**0.0025**	**<0.0001**	**0.0002**	NS	**0.0043**	ns	**<0.0001**	**<0.0001**	**<0.0001**	**0.0031**
Interactions										NS	
YR x IR	**0.0056**	NS	NS	NS	**0.0476** [5]	NS	**0.0115** [6]	NS	-	NS	-
YR x FT	NS	NS	**0.0054** [1]	NS	**0.0009** [2]	**0.0158** [3]	**0.0038** [4]	NS	-	NS	-
IR x FT	NS	0.075	NS	NS	NS	0.0514	NS	NS	NS	NS	NS
YR x SV	**<0.0001** [7]	**0.0010** [8]	NS	**0.0181** [9]	0.0879	**<0.0001** [10]	NS	NS	-	NS	-
IR x SV	**0.0015** [11]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
FT x SV	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
YR x IR x FT	**0.0265** [12]	**0.0211** [12]	NS	NS	NS	0.0568	NS	NS	-	NS	-

Means that are followed by the same letter within each column are not significant different (general linear hypothesis test $p<0.05$)

*CHI: chicken manure; *MIN: mineral fertiliser; *SHE: sheep manure;

[1]See figure 5.4.1 for interaction means ±SE; [2]See figure 5.4.2 for interaction means ±SE; [3]See figure 5.4.3 for interaction means ±SE; [4]See figure 5.4.4 for interaction means ±SE; [5]See figure 5.4.5 for interaction means ±SE; [6]See figure 5.4.6 for interaction means ±SE; [7]See figure 5.5.7 for interaction means ±SE;[8]See figure 5.5.8 for interaction means ±SE; [9]See figure 5.4.9 for interaction means ±SE; [10]See figure 5.4.10 for interaction means ±SE; [11]See table 5.4.11 for interaction means ±SE; [12]See table 5.4.1 for interaction means ±SE;

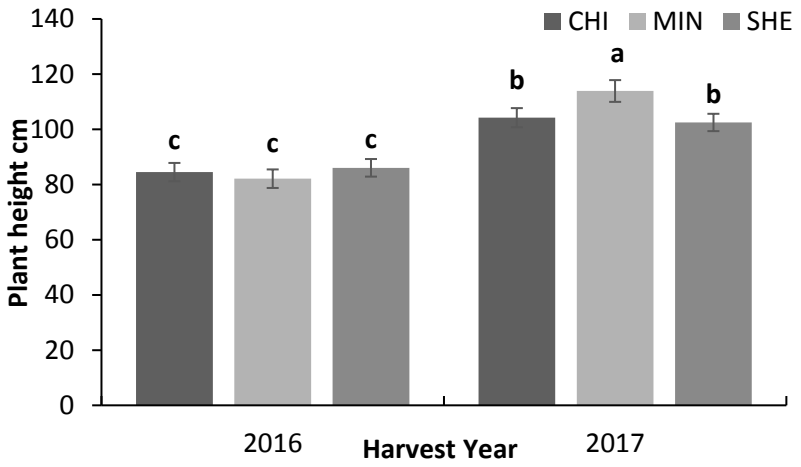


Figure 5.4.1 Effects of interaction between harvest year and fertiliser type on spelt wheat plant height at GS62.

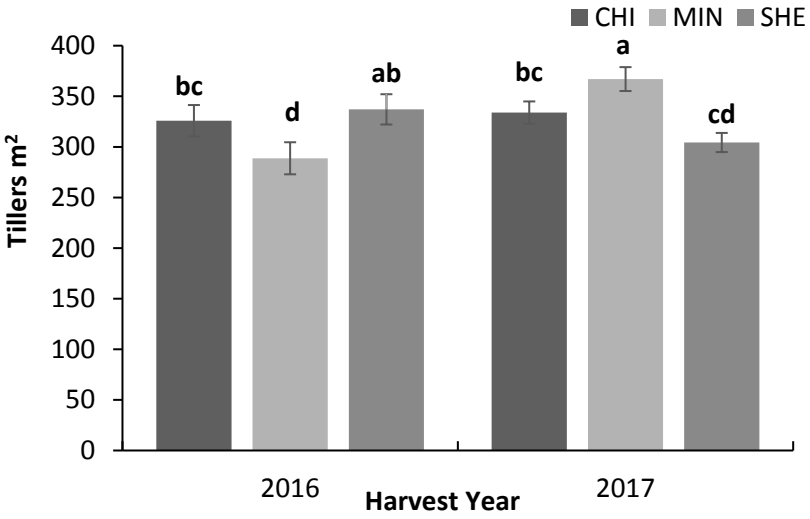


Figure 5.4.2 Effects of interaction between harvest year and fertiliser type on spelt wheat number of tillers at GS85.

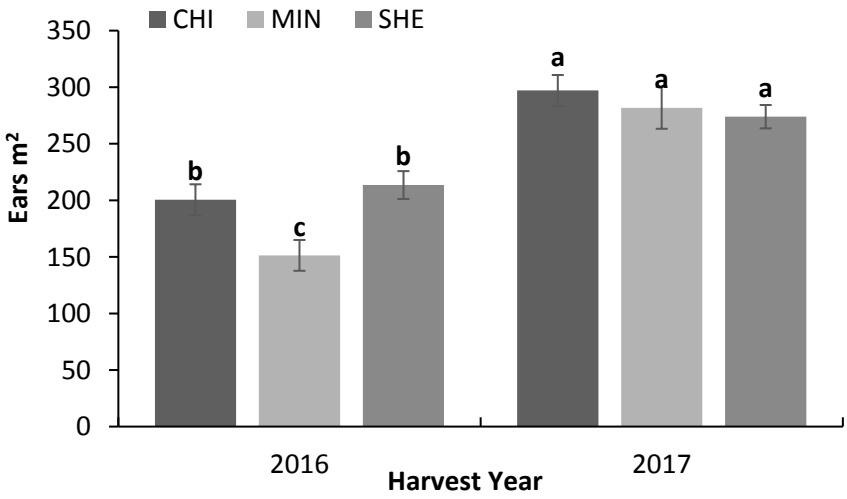


Figure 5.4.3 Effects of interaction between harvest year and fertiliser type on number of ears of spelt wheat at GS85.

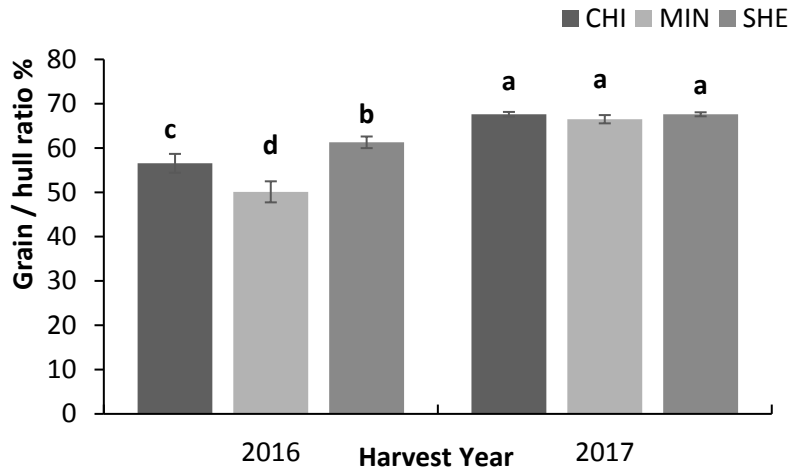


Figure 5.4.4 Effects of interaction between harvest year and fertiliser type on spelt wheat grain to hull ratio.

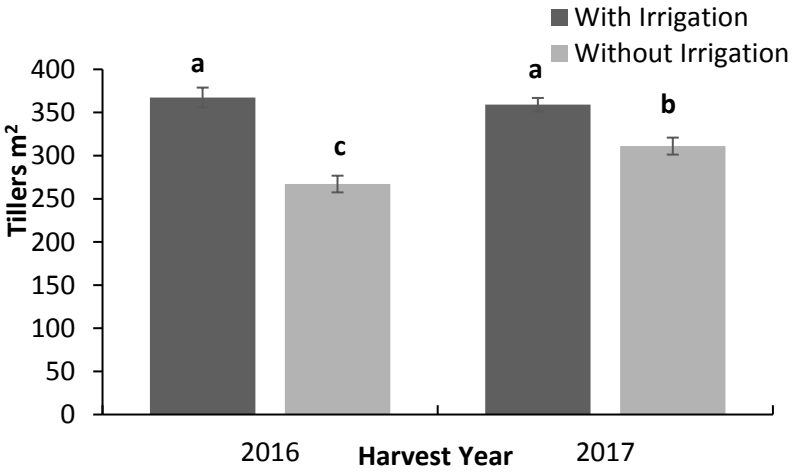


Figure 5.4.5 Effects of interaction between harvest year and irrigation on spelt wheat number of tillers at GS85.

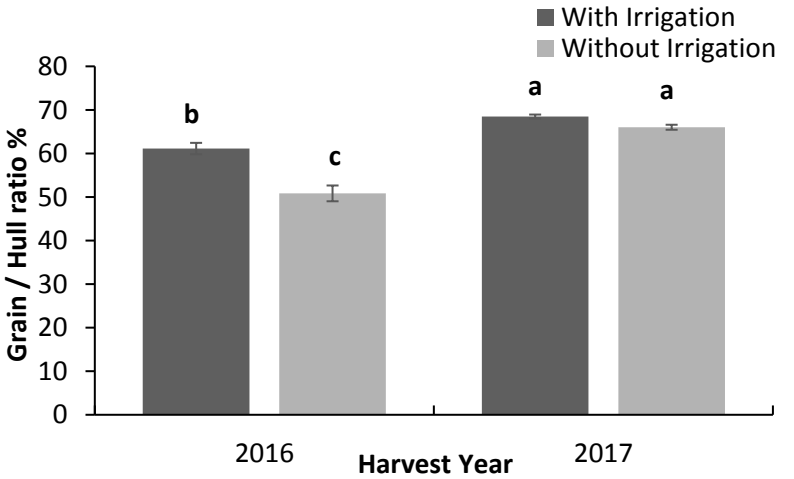


Figure 5.4.6 Effects of interaction between harvest year and irrigation on spelt wheat grain to hull ratio.

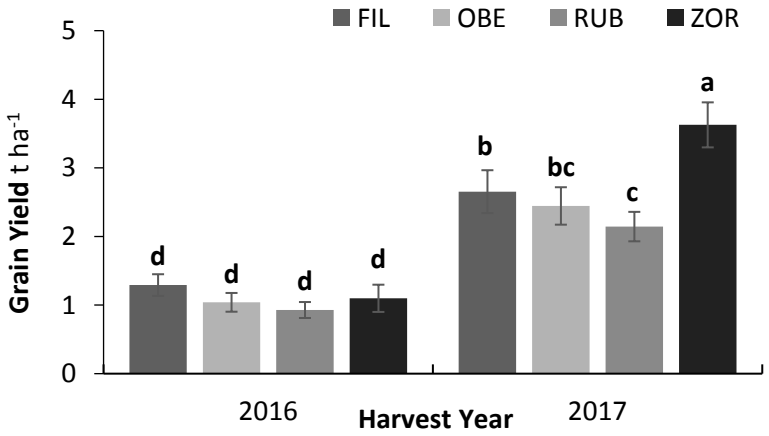


Figure 5.4.7 Effects of interaction between harvest year and variety choice on spelt wheat grain yield

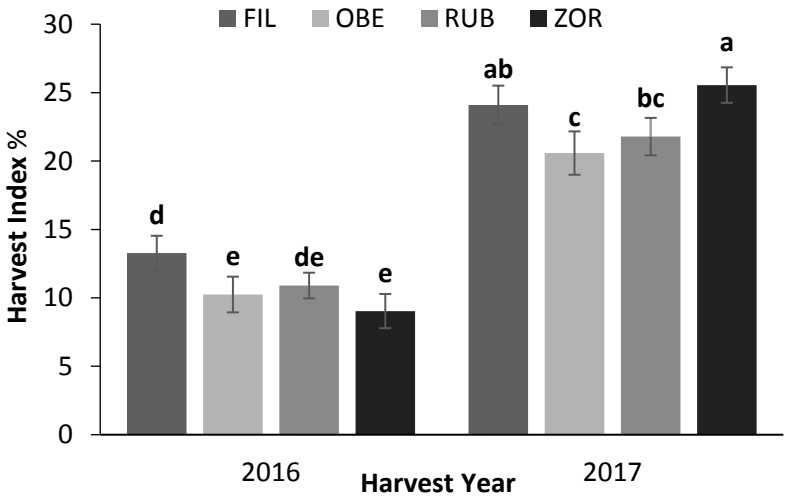


Figure 5.4.8 Effects of interaction between harvest year and variety choice on spelt wheat harvest index

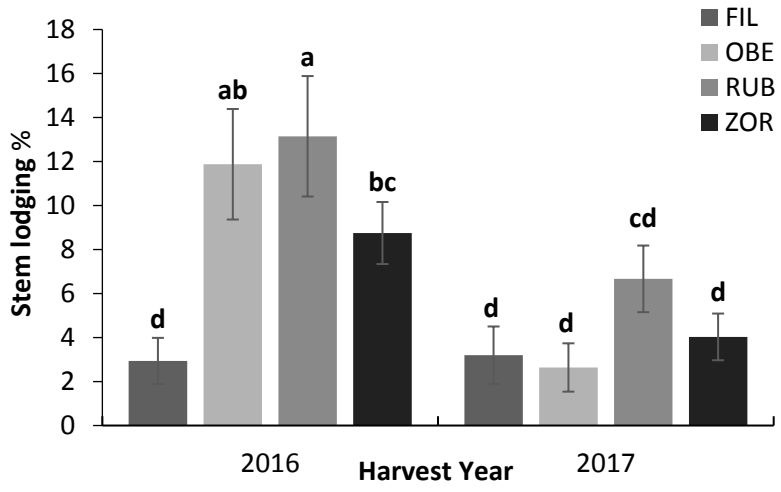


Figure 5.4.9 Effects of interaction between harvest year and variety choice on spelt wheat stem lodging.

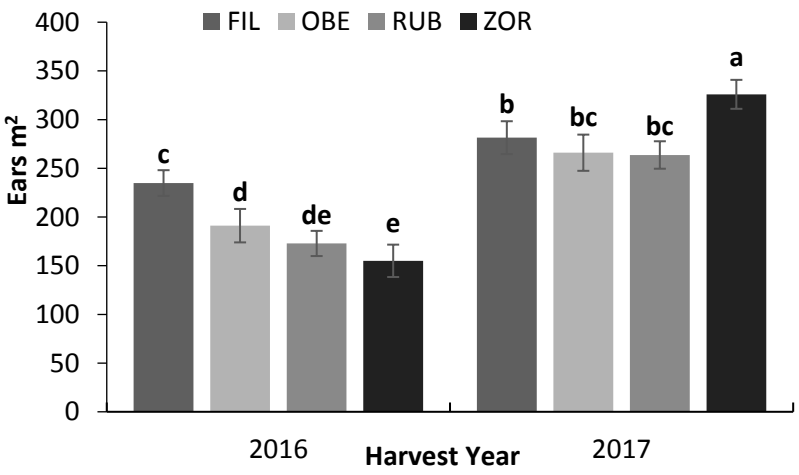


Figure 5.4.10 Effects of interaction between harvest year and irrigation on number of ears of spelt wheat at GS85.

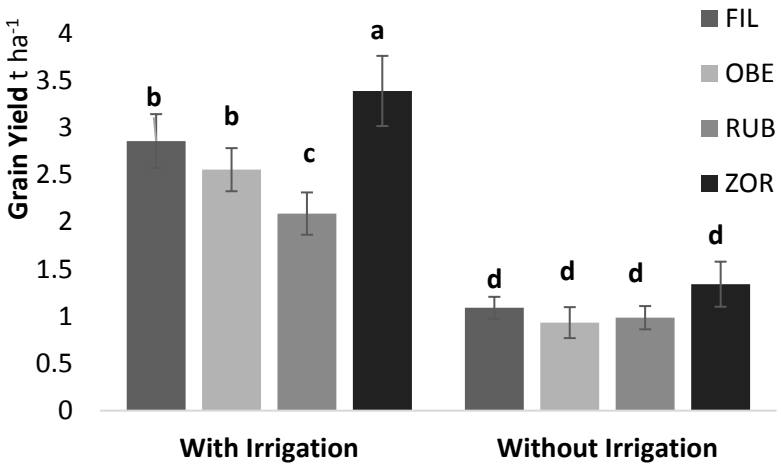


Figure 5.4.11 Effects of interaction between irrigation and variety choice on spelt wheat grain yield

Table 5.4.1 Interactions means ± SE for the effects of harvest year, irrigation and fertiliser types on grain yield and harvest index.

Parameter	Factor 1 **Year**	Factor 2 **Irrigation**	Factor 3 **Fertility Types** CHI*	MIN*	SHE*
			t/ha		
Grain Yield	2016	+irrigation	1.70 ±0.13 **A b**	1.20 ±0.16 **B b**	1.86 ±0.18 **A b**
		-irrigation	0.57 ±0.09 **A c**	0.40 ±0.13 **A c**	0.80 ±0.09 **A c**
	2017	+irrigation	4.01 ±0.27 **A a**	3.95 ±0.29 **A a**	3.60 ±0.26 **A a**
		-irrigation	1.85 ±0.23 **A b**	1.10 ±0.17 **B b**	1.80 ±0.16 **A b**
			%		
Harvest Index	2016	+irrigation	15.17 ±1.43 **A c**	9.87 ±1.14 **A b**	15.15 ±1.28 **A b**
		-irrigation	8.80 ±1.54 **AB d**	5.79 ±1.10 **B c**	10.40 ±1.02 **A c**
	2017	+irrigation	27.31 ±0.79 **A a**	24.89 ±0.82 **A a**	27.72 ±0.74 **A a**
		-irrigation	21.64 ±1.93 **A b**	11.72 ±1.43 **B b**	24.74 ±0.83 **A a**

*CHI: chicken manure; *MIN: mineral fertiliser; *SHE: sheep manure;
For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (Tukey's honestly significant difference test P<0.05)

5.4 Discussion

5.4.1 The effect of fertilisers with contrasting water-soluble N and P release/availability characteristics on crop health, grain yield and quality parameters

Mineral fertilisation significantly increased grain protein contents, but had no substantial overall impact on the mineral and antioxidant concentrations in spelt grains (Tables 5.1 to 5.3). This is consistent with studies with common wheat (Bilsborrow *et al.*, 2013; Hlisnikovský and Kunzová, 2014).

The results of the SPAD measurements (which provide an estimate of N-availability) indicate that the use of mineral NPK fertiliser increased N-supply to crops, which is known to increase grain protein levels, which was consistent with several previous studies showing that additional N-fertilisation at the beginning of ear development (GS 51) increased the protein content of the grain (Mäder *et al.*, 2007a; Zörb *et al.*, 2009; Vrcek *et al.*, 2014).

The finding that mineral fertilisation has no substantial overall impact on the mineral concentration/profiles in spelt (Tables 5.1 and 5.2), which was consistent with the study by Saha *et al.* (2010) and the meta-analysis of comparative (organic vs conventional) food composition data by Baranski *et al.* (2014). In contrast, the retail survey presented in Chapter 4 (Tables 4.5 and 4.6), and previous studies reported that significant higher concentrations of macro and micro minerals were detected in organic compared with conventional cereals (Worthington, 2001a; Gomez-Becerra *et al.*, 2010a; Gomez-Becerra *et al.*, 2010b; Hussain *et al.*, 2010; Lairon, 2010b; Baranski *et al.*, 2014). It is important to highlight that although no significant differences between fertiliser types were detected for any of the toxic metals assessed in this study, concentrations of cadmium (Cd) were numerically lower in grain from mineral fertilised plots; this contradicts the results of the meta-analysis of comparative data by Baranski *et al.* (2014) and a recent field experimental study by Cooper *et al.* (2013) which both reported that Cd concentrations are substantially higher in mineral fertilised conventional cereals compared with organic cereals. However, in this study much lower mineral P (40 kg ha^{-1}), the main source of Cd inputs into soils, was used than in most previous studies with common wheat, where P-inputs were at least twice as high (Rempelos *et al.*, 2018b).

In the present study the use of organic fertilisers resulted in slightly lower total phenolic concentrations than mineral fertilisers, while there was no significant difference between fertiliser treatments for total flavonoid concentrations and antioxidant capacity in grains (Table 5.3). This is consistent with some previous studies (Saha *et al.*, 2010), but different to **(a)** the results of the meta-analysis by Baranski et al. (2014), (b) the retail survey of wheat flour reported in Chapter 4 and **(c)** a range of other previous studies (Zuchowski *et al.*, 2011; Fares *et al.*, 2012; Konopka *et al.*, 2012b; Kesarwani *et al.*, 2013; Kesarwani *et al.*, 2014; Legzdina *et al.*, 2014), which all reported significantly higher concentrations of phenolic compounds

and/or antioxidant capacity in organically than conventionally produced cereals. In several studies differences in antioxidant concentrations were linked to contrasting levels of pest and disease damage in organic and conventional farming systems (Nicholson and Hammerschmidt, 1992; Bennett and Wallsgrove, 1994; Varga *et al.*, 2012; Almuayrifi, 2013; Baranski *et al.*, 2014). However, in this experiment, only different fertiliser types were compared and pesticides were not applied to the plots receiving conventional mineral fertiliser inputs. Therefore, the incidence of pest and disease damage for organic and conventional treatment plots were similar. This may therefore have been a potential reason why no significant difference in antioxidants content between organic and conventional treatment (organic vs conventional fertiliser) were detected. However, it should be pointed out that in a recent factorial experimental study **(a)** the severity of fungal diseases caused by obligate pathogens (mildew and rust) was higher in conventional than organically managed cereal crops; and **(b)** only the different fertiliser (but not crop protection) regimes used in organic and conventional farming had a significant effect on phenolic acid and flavonoid concentrations in flag leaves of common wheat (Rempelos et al. 2018).

Previous studies (Tétard-Jones *et al.*, 2013; Rempelos *et al.*, 2018a) with common wheat showed that the use of NPK fertiliser results in higher crop yields and higher lodging severity than manure applied at the same N-input level (Table 5.4), and that this was at least partially due to the higher nutrient (especially N) availability from mineral fertilisers. It was therefore expected that spelt wheat would show similar trends.

The higher lodging severity in mineral NPK fertilised plots found in the current study with spelt wheat was therefore consistent with the results of previous studies. However, the finding that chicken and sheep manure resulted in similar or higher grain yields and other performance/growth parameters (including harvest index and TGW) was surprizing (Table 5.4), especially since (a) SPAD measurements in this study indicated higher levels of N-supply in minerals compared with compost fertiliser plots and (b) previous studies with common wheat had also shown that the proportion of plant available soluble N is higher from mineral compared with animal manure inputs (Bilsborrow *et al.*, 2013).

One explanation may be, that nutrient losses due to leaching and especially run-off may have been higher in mineral NPK fertilised plots, since (a) the water soluble minerals N and P are more prone to losses than the organic forms of N and P present in manure and (b) there is substantial run-off during the heavy winter/spring rainfall events typical for Crete. Another explanation may be that the high temperature in the Messara regions could increase the microbial community activity and increase the speed of mineralization of organic manures and

compost, which resulted in similar rates of N-utilisation to mineral fertiliser (Whitmore, 2007). Also, the nutrient supply required to achieve the yield potential of spelt wheat may be lower than those of modern common wheat varieties, and may therefore have been provided by manure inputs, while mineral NPK fertiliser inputs provided an excessive nutrient supply especially in the two drier years, where water was the main yield limiting factor. This view is supported by the interaction between irrigation and fertiliser type where sheep manure delivered the highest yield in the drier year, while mineral fertiliser resulted in higher yields than the 2 manure treatments in the wetter year. This view is also supported by a previous study which indicated that common wheat growth and performance was less affected by differences in rainfall/irrigation than wheat grown with conventional fertiliser when rainfall/irrigation is limited (Kitchen *et al.*, 2003).

Overall, the results from this study may suggest that the use of sheep manure is the most suitable fertiliser for spelt production in the Messara regions, since it produces similar yields to the other two fertilisers, but does not increase stem length and associated higher lodging risk; however this should be confirmed in future studies.

5.4.2 The effect of supplementary irrigation on grain yield and nutritional quality parameters of rain-fed winter spelt wheat

Spelt wheat, a crop which has until now been grown mainly in more temperate regions of Europe is usually grown without supplementary irrigation. As expected from previous studies with common wheat (Guttieri *et al.*, 2005; Ma *et al.*, 2015), supplementary **irrigation**, increased grain yields, harvest index, straw length, but also the severity of lodging. However, this is, to our knowledge, the first study in which the effect of irrigation on nutritional composition parameters has been determined. The finding that irrigation increased grain yields without resulting in a substantial effect on mineral composition, phenolic/flavonoid content and antioxidant capacity is therefore particularly important, because it demonstrates that supplementary irrigation allows spelt wheat productivity to be increased without a reduction in nutritional value (Tables 5.1 to 5.4).

The finding that supplementary irrigation had no substantial overall impact on mineral and phenolic concentrations and antioxidant capacity (Tables 5.1 to 5.3) was different to results reported for common wheat and/or other cereals. For example, two recent studies showed that the mineral contents in the grains of common wheat were greatly affected by soil water availability (Zhao *et al.*, 2009a; Singh *et al.*, 2012). This could indicate that spelt wheat has a greater nutrient and possibly water stress resistance than modern common wheat varieties and should be investigated in future studies. Previous studies with common wheat have also reported higher total phenolic concentration and/or antioxidant capacity in grains from common wheat crops under water stress and/or grown with lower water input levels from irrigation (Helyes *et al.*, 2012; Martinelli *et al.*, 2012; Wu *et al.*, 2017). Similarly, Varga *et al.* (2012) reported higher concentrations of free, bound and total phenolic compounds with deficit irrigation than full irrigation among all sorghum genotypes tested in their study. This study concluded that this was due to water stress and/or changes associated with water stress (e.g. nutrient deficiencies and insect attack) affecting either phenylalanine ammonia-lyase (PAL) synthesis or PAL activity in plants, which is an essential enzyme in the pathway of phenolic synthesis (Varga *et al.*, 2012). However, it should be pointed out that, (a) long-term drought has also been shown to cause a reduction in the antioxidant capacity and total phenolic content in cereals in some other studies (Hura *et al.*, 2008; Khosh-Khui *et al.*, 2012; Saharkhiz Mj, 2012; Ma *et al.*, 2015) and (b) temperature is also a very important factor affecting the phenolic/antioxidants concentration in crops (Bita and Gerats, 2013). The latter, may be an explanation, why phenolic concentrations were lower in 2016 (the year with exceptionally low rainfall coming with higher temperature) than 2017 (the higher rainfall year coming with lower temperature) while there was no significant effect of supplementary irrigation.

Although no substantial effect on mineral and phenolic concentrations could be detected, supplementary irrigation significantly reduced the grain protein content, irrespective of the variety used (Table 5.1) and this is known to affect processing quality especially for bread making. The finding that supplementary irrigation reduce grain protein content is consistent with previous studies in common wheat (Guttieri *et al.*, 2005; Zhao *et al.*, 2009a; Singh *et al.*, 2012). For example, Zhao *et al.* (2009a) showed that when soil water content increased from 45% to 85%, grain yields increased, but the grain protein contents decreased from 16.7% to 14.8%. Therefore, it may be important to investigate the effect of irrigation schedules which ensure sufficient water supply during the earlier vegetative growth stages but generate a slight water deficit post-anthesis stage, to allow an increase in grain yield without substantial reductions in protein content to be achieved as previously suggested (Zhao *et al.*, 2009a). Supplementary irrigation is thought to affect protein content and protein to carbohydrate ratios in wheat grain primarily by affecting N-supply and redistribution in the plant, and it may therefore be worthwhile to investigate the effects of using precision fertilisers which increase protein content in irrigated production systems via increasing N-availability at specific growth stages (Guttieri *et al.*, 2005; Singh *et al.*, 2012).

Results of the study reported here showed that irrigation only increased yields in the two years with low (2016) and moderate (2017) rainfall (Table 5.4), but not in 2015 the year with relatively high rainfall (Appendix 5.1). This suggests that the use of irrigation will primarily deliver yield stability over time, but will only result in a substantial additional yield in years with low rainfall. In the year with high rainfall, supplementary irrigation resulted in substantially higher lodging, which is known to have a negative impacts on grain quality (e.g. higher mycotoxin and lower grain protein levels). This suggests that supplementary irrigation should be managed carefully and only applied when monitoring of soil moisture levels and weather forecasts predict that it will be beneficial for crop growth. These results are consistent with those of a previous study with common wheat by Sun *et al.* (2006) which found that in years with limited rainfall, irrigation can significant increase the yield of wheat, but that excessive amount of irrigation can decrease grain yield and water use efficiency.

5.4.3 The effect of variety choice (spelt genotypes from contrasting breeding programmes and selection backgrounds) on crop health, grain yield and quality parameters

Variety choice had the greatest impact on quality parameters and yield in spelt wheat and emphasised the importance of site and production-specific development of new varieties (Table 5.4).

Results from this experiment showed that under semi-arid conditions, the “organic” spelt variety ZOR (which was recently developed specifically for the organic arable sector) and the “modern” spelt variety FIL (which is based a *T. aestivum* x *T. spelta* cross) and was developed for the conventional arable sector) produced higher grain yields and numbers of ears.

The finding that FIL had both the highest grain yield and phenolic and flavonoid concentrations but the lowest lodging severity suggests that it is the most suitable variety for organic production in semi-arid regions. This is because FIL may allow farm profits to be increased through higher yields (without any extra costs) and through nutritional quality (high antioxidant content) focused marketing, which may allow additional price premiums to be achieved especially in the organic sector. The results suggest, that concerns about “modern” short-straw spelt varieties (based on spelt x common wheat crosses) having a lower nutritional value may be unfounded and that such crosses may even improve the nutritional value of spelt. This may be due to there being a higher degree of polymorphism between wheat and spelt (Messmer *et al.*, 1999) and/or possible “heterosis” effects in wheat-spelt crosses (Schmid and Winzeler, 1990). The low lodging potential also makes this variety the most suitable variety for production systems in which grain yields are increased through supplementary irrigation. The protein content achieved by FIL may still be sufficient for baking quality, especially the sourdough, long-fermentation based baking systems that are increasingly popular in the organic sector, but this needs to be confirmed in baking tests.

The pure spelt wheat varieties RUB and OBE had higher lodging severity, which can result in lower yields and grain quality (e.g. through infection by mycotoxin-producing fungi). These traditional spelt varieties also had lower antioxidant levels than both modern varieties (FIL and ZOR). Based on the results from this study, it is therefore difficult to justify the current “higher nutritional value” focused marketing of traditional spelt varieties, although they were confirmed to have 5-20% higher Mn, Cu, Fe and Zn concentrations in grain. Due to their greater susceptibility to lodging, the traditional spelt varieties included in trials are also unsuitable for irrigated production in semi-arid regions such as Crete. However ZOR and FIL had significantly lower protein concentrations than traditional RUB and OBE, and if the modern varieties are shown to not have the baking quality characteristic desired by bakers then traditional spelt varieties may still be required to improve the protein content of spelt flour. This

may be particularly important for modern short fermentation baking processes, which are known to require higher protein contents. The negative correlation between yield and protein content in contrasting varieties was previously linked by environmental factors, source-sink interactions and dilution of protein by non-protein compounds (Kibite and Evans, 1984). However, previous studies examining heterogeneity for protein content in spelt (based on benchmarking from 772 Spelt wheat genotypes indicated substantial levels of variation (some genotypes producing up to 30% protein) for both mineral and protein content and the potential to increase both yield and protein levels through breeding approaches (Gomez-Becerra *et al.*, 2010a). A major objective of future spelt wheat breeding and selection programs should therefore be improving protein quality and or mineral content.

5.5 Conclusions

Overall, under semi-arid conditions, especially in dry years, supplementary irrigation was shown to substantially improve grain yield of spelt wheat (especially if modern spelt varieties with greater lodging resistance were used) but this had no negative effects on minerals and phenolic/antioxidant concentrations. Sheep and chicken manure fertilisation resulted in similar yields to mineral fertilisation which emphasised the suitability of spelt for organic production. Fertiliser type did not have a major impact on mineral and phenolic/antioxidant concentrations in grains, but this should be confirmed in further long-term experiments. To improve yield, yield stability and grain quality, breeding/variety selection was shown to be a very important strategy to improve spelt wheat performance. The study also demonstrated the importance of variety selection under target climatic and agronomic background conditions as previously recommended by van Bueren *et al.* (2011).

Chapter 6 Main achievements

Due to the complex design and the large amount of outcomes of the project, findings which contribute to the science development along with the importance of the work are emphasized in this brief chapter.

6.1 Organic agronomic practices contributing to the reduction of pesticides contamination and the enhancement of nutrition in cereals

Consumers are increasingly aware about food safety issues and demand safe and nutritious food with minimal microbiological or chemical contaminants (Bhat *et al.*, 2010), which contributed to the increase in the demand for organic food products (Wier *et al.*, 2008; Yadav and Pathak, 2016). This project confirmed that there are significantly higher levels of total phenolic content, total antioxidant capacity, micronutrients Fe and Zn in organic than conventional wheat flour (Figure 6.1). These results indicated that switching to organic from conventional cereals enables 11%, 16% and 30 % more phenolics, micronutrients Fe and Zn intake respectively when consuming the same amount of grains/flour. The recommended intake of Fe and Zn for adults between 19-50 years old is, respectively, 14.8 and 9.5 mg (BDA, 2017; NHS, 2017). Based on this study It can be estimated that consuming 100g of organic wheat per day can supply 15% and 17% of the average daily intake requirement for Fe and Zn while conventional flour would supply 13% and 12%.

The results bring together the most up to date findings for German and UK markets that switching to organic cereal production methods will result in a 10-fold reduction in CCC, a 4-fold reduction in total CPP residues and an 8-fold reduction in the number of wheat samples contaminated with multiple CPP residues including deltamethrin, chlormequat, piperonyl butoxide and pirimiphos-methyl (Figure 6.2). The results clearly demonstrate the large and significant difference in levels of pesticides residues between organic and conventional products and emphasize the multi-residue problem of pesticide in cereals, which has previously been ignored and only investigated in few studies about the correlation between total pesticide contamination and public health (Lydy *et al.*, 2004; Gaw *et al.*, 2008; Parrón *et al.*, 2011; Hernández *et al.*, 2013). In the majority of cases, the concentrations of pesticides in food do not exceed the legislatively determined safe levels (EFSA, 2018). However, these “safe limits” may underestimate the real health risk as in the case of simultaneous exposure to two or more chemical substances, which occurs in real-life conditions and may have synergistic effects (Jeyaratnam, 1990; Kortenkamp, 2007). EFSA (2018) cannot present results of cumulative risk assessment yet as the scientific preparatory work is not completed (EFSA, 2018). However, many epidemiologic evidences clearly had shown that at current

exposures pesticides adversely affect human health including increasing cancer risks and neurotoxicity (Alavanja *et al.*, 2004; Bassil *et al.*, 2007). Therefore, it is suggested by Abdollahi *et al.* (2004) and Bassil *et al.* (2007) that the pesticide residues in food and environment should be reduced, which can be achieved by switching to organic cereals and organic farming system as indicated by this study.

6.2 The DON and OTA contamination in cereals and their changes over time

The meta-analysis carried out for this thesis is, to the author's knowledge, just the second study to be carried out comparing mycotoxin contamination between organic and conventional cereals. However, the current meta-analysis contains 7 times more data points, than the previous analysis (Smith-Spangler *et al.*, 2012a), resulting in 20% lower heterogeneity (I^2 value) in the outcome measures. This lower heterogeneity equates to greater certainty in the outcomes reported which indicated that organic cereals were contaminated significantly less with the *Fusarium* mycotoxin DON than conventional cereals and organic cereals were contaminated with higher levels of OTA than conventional cereals, but that the contamination and prevalence of OTA has decreased in cereal grains/products in the last 15 years in Europe (Figures 6.3 and 6.4).

This result that DON contamination is higher in organic than conventional cereals, however, is contrary to a commentary in Nature (Trewavas, 2001b), which suggested that organic farming practices result in higher levels of *Fusarium* mycotoxin contamination in crops. This information from 2001 was widely cited in the scientific literature and public media, raising considerable concerns among consumers about the safety of organic food. Therefore, the opposite finding reported here is an important contribution to the debate and may bring new consideration to the science. In addition, it identifies a new approach/idea for reducing mycotoxin contamination, which is achieved through agronomic practice management. However, the specific agronomic reducing the *Fusarim* mycotoxin contamination need to be further investigated by studies. Changing production systems is especially important given the current situation where there has been increasing food losses resulting from mycotoxin contamination in the last decade as a problem associated with global warming, and when there is increasing epidemiological evidence for harmful effects of mycotoxins on both human and animal health (Bryla *et al.*, 2016; Ferrigo *et al.*, 2016).

Fusarium mycotoxin DON mentioned above is primarily affected by environmental conditions and agronomic practices pre-harvest, while contamination with common mould mycotoxins is more affected by post-harvest grain treatment, storage and quality assurance practices. OTA has been shown to be a consequence of insufficient post-harvest drying of grain and poor

grain storage conditions (Magan and Aldred, 2005; Magan and Aldred, 2007; Magan *et al.*, 2010; HGCA, 2014), especially prior to 2004 in organic farms with poor grain drying and/or storage facilities (Jorgensen and Jacobsen, 2002; Köpke *et al.*, 2007). Therefore, results of mycotoxin levels reported there could indicated that the reduction of OTA contamination in cereals is mainly due to improvements in postharvest treatments and quality assurance procedures seen in the organic farming sector.

Despite the decreasing tend for OTA, the findings from the supermarket shopping basket study reported in this thesis show that OTA concentrations were close to, and for a substantial number of samples above the current MCL set (3 µg/kg) by the EU (Figure 6.6). This surprising result is an important finding which should be of concern and lead to a requirement for further improvements in post-harvest drying, storage, quality assurance facilities/protocols (e.g. introduction of detailed mycotoxin testing) and storage management in supermarkets (which is often ignored) for cereal flours, especially for organic products to minimise the OTA contamination, because OTA has been found to be a potent renal toxin in all of the animal species tested. The extent of renal injury is dose-dependent, but is also associated with the duration of exposure, as OTA accumulates in renal tissue(European Food Safety Authority, 2006). On the basis of the lowest observed adverse effect level of 8 µg/kg body weight per day for early markers of renal toxicity in pigs, a tolerable weekly intake of 0.12 µg/kg body weight was derived for OTA after applying a composite uncertainty factor for the uncertainties in the extrapolation of experimental data derived from animals to humans. Recent analyses of the dietary exposure of adult European consumers to OTA revealed that at present the weekly exposure ranges from 0.015 to 0.06 µg OTA per kg body weight per week, which is below the recommended 0.12 µg/kg bodyweight per day. However, as current EFSA consumption databases do not include infants and children (European Food Safety Authority, 2006) this needs to be considered further.

Furthermore, the significant differences of OTA contamination between flour from German and UK are likely to be due to the difference of (a) the climate and (b) the storage management from the farm to the retailers in the two countries. The significance of the differences observed is unclear, and whether these differences are observed across more than two years' of analysis needs further investigation.

6.3 The importance of spelt variety selection under target climatic and agronomic background conditions, and its nutrition advantages compared with common wheat

Spelt wheat (*Triticum spelta*) is currently increasing its share of the cereal market (Escarnot *et al.*, 2012). Being a 'robust' cereal, it is widely known that spelt wheat is well adapted to cool and wet pedo-climatic conditions of higher altitudes (Rüegger and Winzeler, 1993), and to our knowledge, it has not been previously grown in semi-arid regions of the Mediterranean. As a result, there is no agronomic knowledge with respect to optimum spelt varieties, fertility management and irrigation (especially the drip irrigation which is sustainable) in those regions, which are the most important yield and quality limiting factors. This thesis reports the results of the first experiment to comprehensively explore these factors under controlled experimental conditions in a semi-arid region of the Mediterranean. The field trial indicated drip irrigation increased grain yields of spelt wheat especially in dry years without negative effects on mineral composition, phenolic/flavonoid content and antioxidant capacity. Therefore, the balance between cost of the irrigation system and the profit from increased yield by the irrigation application is an important aspect considered by farmers for profit maximisation. Furthermore, sheep and chicken manure fertilisation resulted in similar yields to those achieved with mineral fertilisation because of high temperature increasing the microbial community activity and the speed of mineralization of organic manures.

Most importantly, it was found that variety choice had the greatest impact on quality parameters and yield in spelt wheat and emphasised the importance of site and production-specific development of new varieties. The “organic” spelt variety ZOR (a Sativa variety bred for organic production and selected under organic farming conditions by Peter Kunz) and the “modern” spelt variety FIL (a short straw variety based on a cross between spelt and a high yielding German common wheat variety developed by Hohenheim University) produced higher grain yields but significantly lower crude protein, Fe and Zn concentrations than the two traditional varieties RUB (a long straw, traditional Czech spelt wheat variety; not thought to be based on crosses with *T. aestivum*) and OBE (a long straw traditional Swiss variety marketed as a “true” or “pure” spelt wheat containing no common wheat genetics) (Figures 6.7 and 6.8). Field experiments with common wheat showed that modern and old varieties also differ significantly from each other in terms of their micronutrient content, with zinc and iron specifically being lower in modern cultivars (Fan *et al.*, 2008) due to the “yield dilution phenomenon” (Shewry *et al.*, 2016).

The protein content of spelt flour is particularly important for modern short fermentation baking processes, which are known to require higher protein contents. The protein content achieved by FIL and ZOR (Figure 6.8) may still be sufficient for good baking quality, especially for

sourdough, long-fermentation based baking systems that are increasingly popular in the organic sector, but this needs to be confirmed in baking tests. The results presented here and previous studies examining heterogeneity for protein content in spelt indicated substantial levels of variation (some genotypes producing up to 30% protein) for both mineral and protein content and the potential to increase both yield and protein levels through breeding approaches (Gomez-Becerra *et al.*, 2010a). Therefore, breeding/variety selection for target use under target climatic and agronomic background conditions was shown in this field experiment to be a very important strategy to improve spelt wheat performance and generally, taking both yield and grain quality into consideration, the “organic” spelt variety ZOR is recommended under Mediterranean conditions.

Few shopping basket studies have directly compared the composition of common and spelt wheat and there are no studies specifically for Germany and UK markets. Results from the shopping basket study presented in this thesis suggest that spelt flour in Germany and UK markets had significantly higher phenolic phytochemical concentrations and total antioxidant capacity than common wheat. In addition, concentrations of mineral micronutrients Zn (Figure 6.9) were significantly and almost 100% higher in spelt than common wheat. It can be estimated by this study that 100g per day spelt wheat can supply 19% of the average daily recommended intake of Zn while the same amount of common wheat would supply 12% of the Zn requirement. These results confirmed the perception of consumers who choose minor cereals because they believe minor cereals including spelt wheat are more beneficial for their health compared with common wheat (*Triticum aestivum*) (Dean *et al.*, 2007).

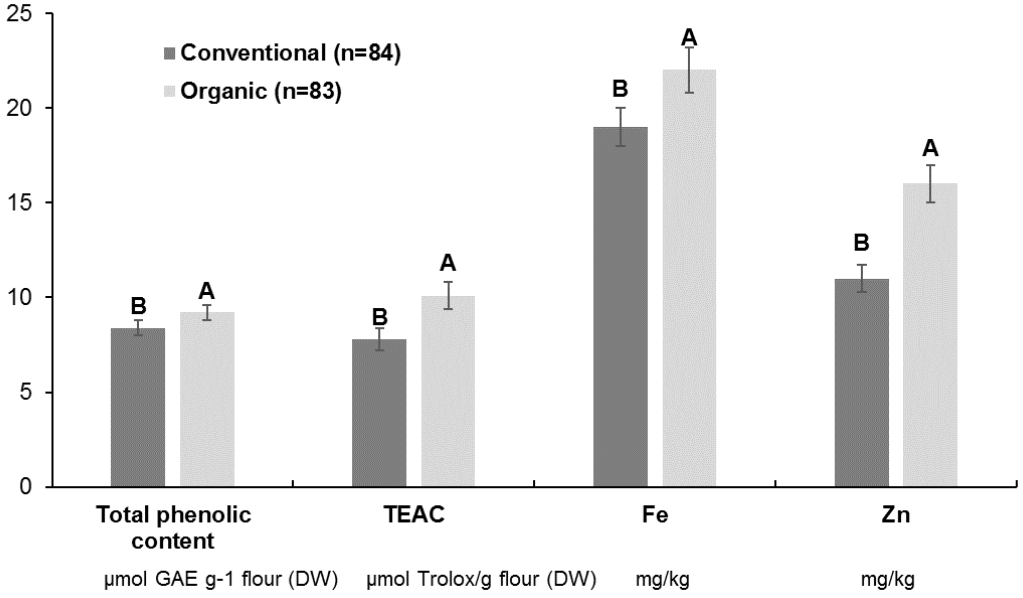


Figure 6.1 Effect of farming system on total phenolic content, total antioxidant capacity by TEAC, Fe and Zn content ±SE in common and spelt wheat flour; For bars labelled with the different capital letter are significant different (P<0.05).

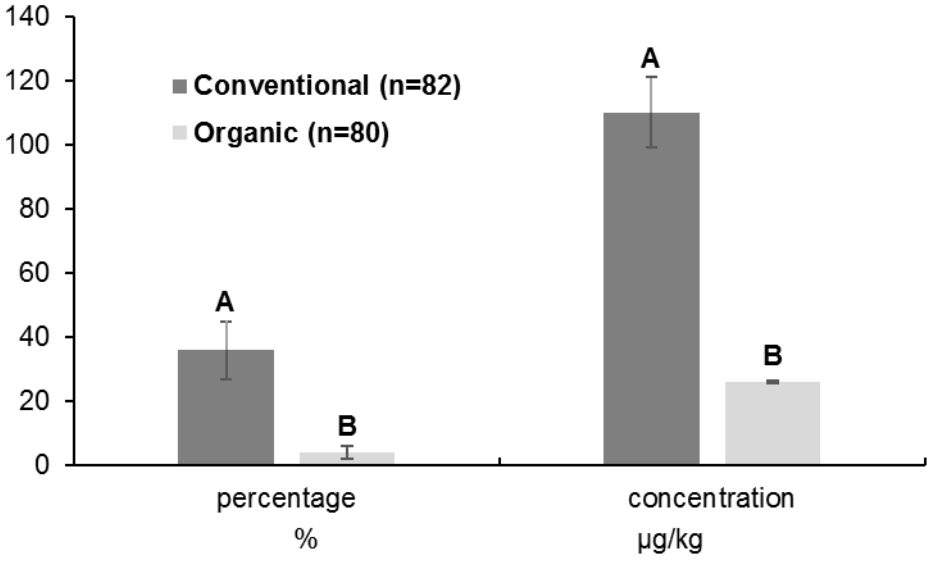


Figure 6.2 Effect of farming system on total concentration ±SE and percentage ±SE of flour samples detected with multiple CPP (sum of deltamethrin, chlormequat, piperonyl butoxide and pirimiphos-methyl) residues; For bars labelled with the different capital letter are significant different (P<0.05).

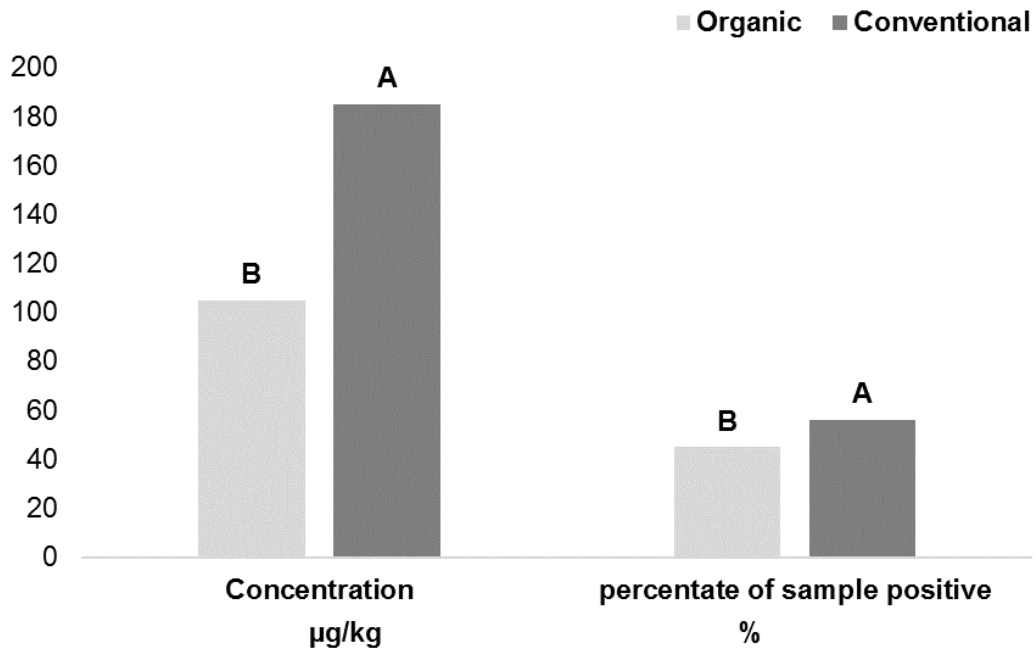


Figure 6.3 Effect of farming system on concentration and percentage of cereal samples contaminated with DON. For bars labelled with the different capital letter are significant different (P<0.05).

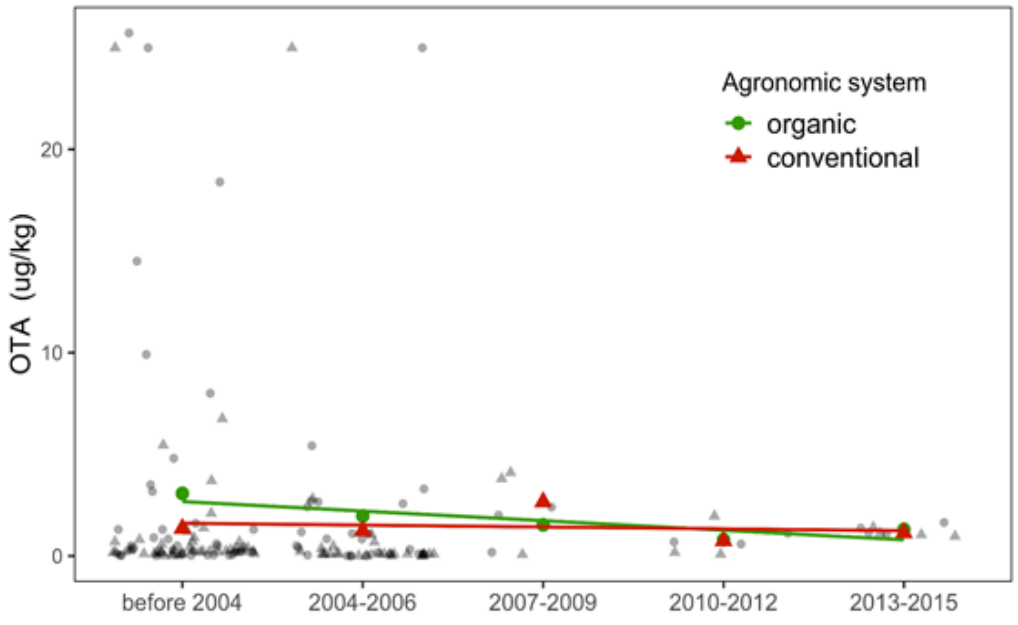


Figure 6.4 Average concentration of OTA found in organic and conventional cereals and cereal products in comparative studies carried out between 1992-2015. The 5 red triangles/5 green dots are averaged concentration of conventional/organic cereal for consecutive 3 year-period since 2004 when EU standard was included for OTA, and grey triangles/grey dots is each date pointe included in the calculation of average concentration for conventional/organic

cereals. The red conventional/green organic regression lines are based on the 5 red triangles/5 green dots.

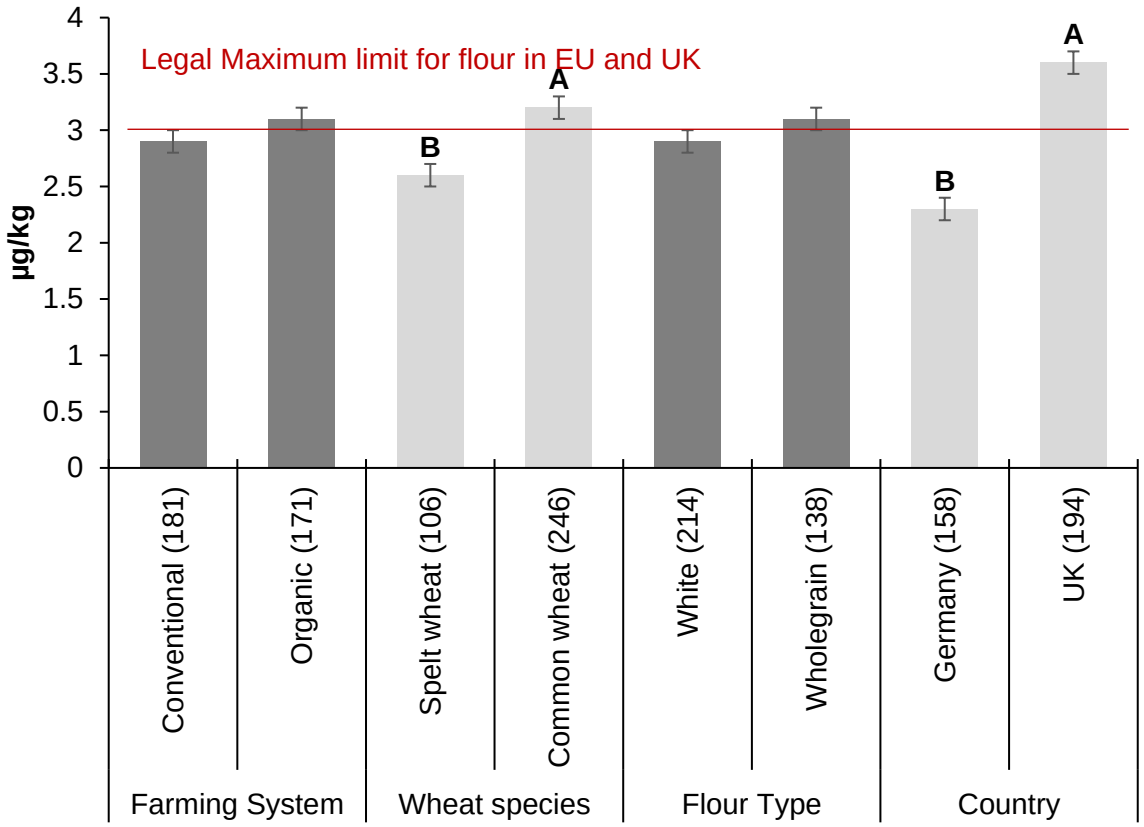


Figure 6.5 Concentration ±SE of OTA found in common and spelt wheat flours between 2015-2017, significant effect of country and species was detected. For bars labelled with the different capital letter are significant different (P<0.05).

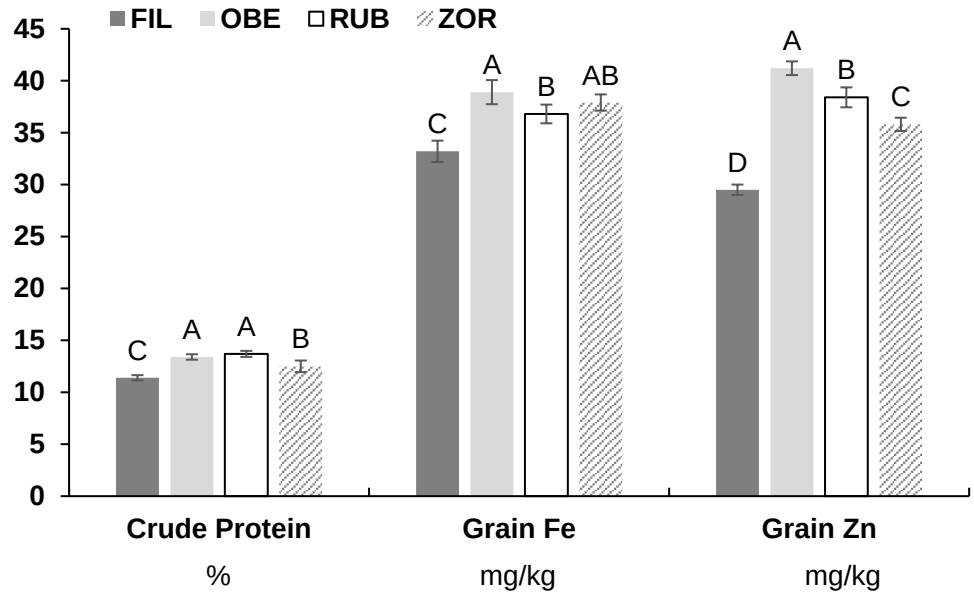


Figure 6.6 Effect of spelt variety on content of crude protein, Fe and Zn in grains in a semi-arid region of the Mediterranean; For bars labelled with the different capital letter are significant different (P<0.05).

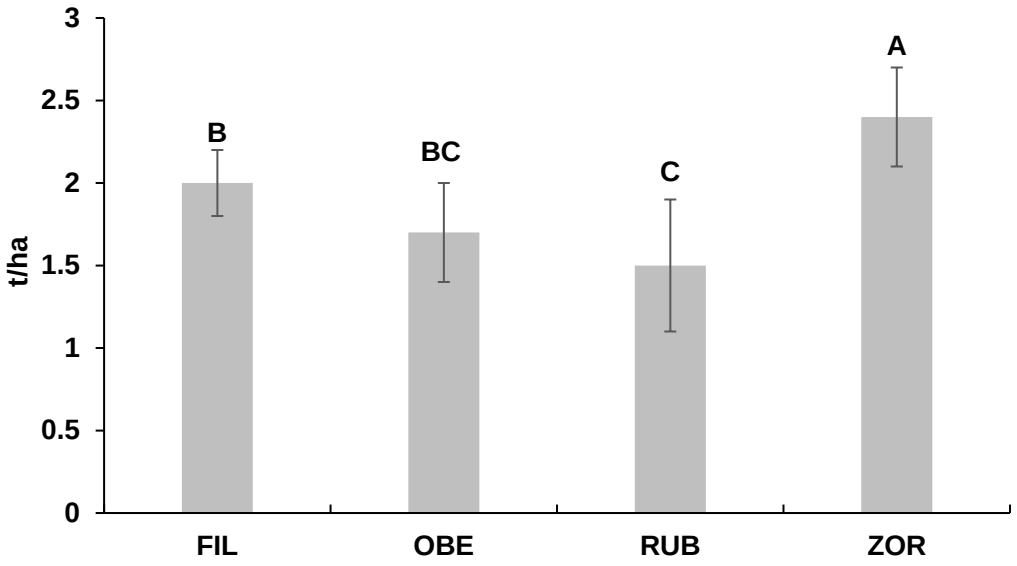


Figure 6.7 Effect of spelt variety on grain yield in a semi-arid region of the Mediterranean; For bars labelled with the different capital letter are significant different (P<0.05).

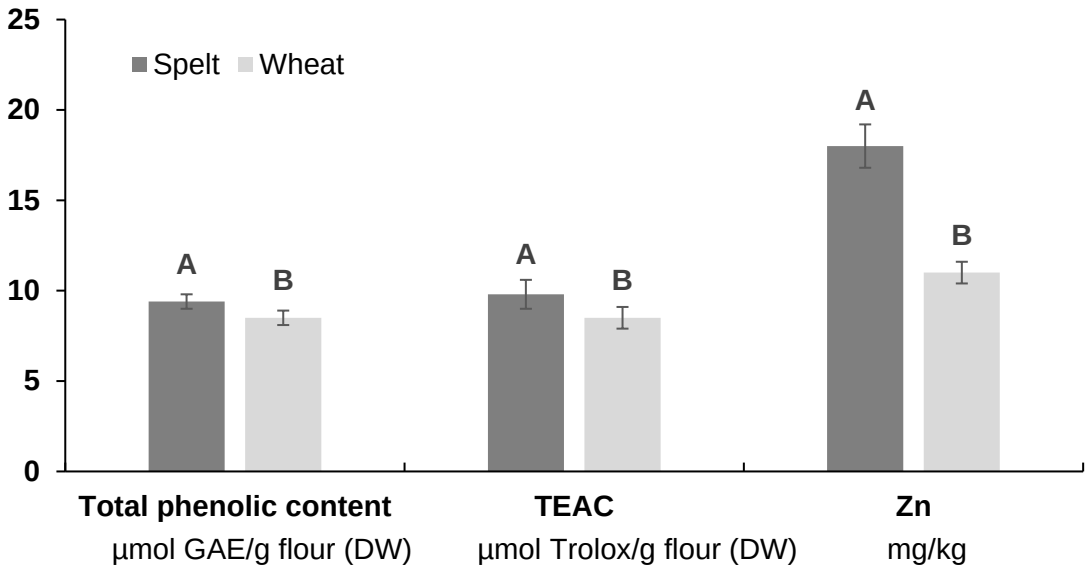


Figure 6.8 Effect of wheat variety on total phenolic content, total antioxidant capacity by TEAC and Zn concentration in common and spelt wheat flour including both wholemeal and white flour. For bars labelled with the different capital letter are significant different ($P<0.05$).

Chapter 7 General discussion and Conclusions

7.1 Undesirable composition in organic vs conventional wheat – results from shopping basket study and meta-analysis

7.1.1 Mycotoxin results from meta-analysis and shopping basket study

Mycotoxin contamination of cereals is a potential threat to human health (WHO 1990). Increasing studies that focused on the mycotoxin contamination in organic vs conventional food have been carried out. However, to our knowledge, only one meta-analysis on mycotoxin comparison in organic and conventional cereals has been conducted (Smith-Spangler *et al.*, 2012a). This analysis showed a higher risk for OTA contamination in organically grown rice but not in wheat compared with conventional alternatives, and also lower levels of DON in organic wheat (Smith-Spangler *et al.*, 2012a). However, it should be pointed out that only 7 or 9 of the primary studies for DON and OTA toxins, respectively, were included in this summary which is not a sufficient body of evidence on which to draw confident conclusions.

The meta analysis reported in this thesis is, to our knowledge, the first one to include more than 50 publications. The results suggest that historically conventional cereals had higher levels of Fusarium mycotoxin contamination, while organic cereals had higher levels of OTA contamination. Results, also suggest that average mycotoxin loads have decreased over time and are now (a) broadly similar in organic and conventional cereals and (b) substantially lower than the maximum levels set by the EC for grains/products destined for adult human consumption.

These results were different to the results of the SBS which indicated that no significant differences in DON, T-2, ZEA and OTA concentrations were found between organic and conventional common and spelt wheat flour examined in supermarket samples collected in the UK and Germany. This may be explained by quality control procedures imposed in the UK and Germany for both conventional and organic grain in the food supply chain. It should be pointed out the SBS indicated that, although not significant, organic flour had numerically higher DON and lower OTA than conventional flour.

As expected, the multilevel meta-analysis model identified cereal species as a major confounding factor for the comparison of mycotoxin contamination in organic and conventional cereal grains/products, but there was insufficient information in the primary publications to include other potential confounding factors such as cereal variety choice, use of irrigation and irrigation method, use of farm-saved seed, and/or whether whole meal or white flour based cereal products were compared. This is a problem for all supermarket basket studies where

label information is limited but these gaps in knowledge should also be investigated in future studies if at all possible.

One particular area of concern is that average concentrations of OTA in both organic and conventional cereal grains/products in the last time period examined (2010 to 2015) were still two times higher than the maximum levels (0.5 µg OTA per kg) set by the EC for cereal-based foods and baby foods for infants and young children. This indicates that there is a requirement for improvements in post-harvest drying, storage, and quality assurance facilities/protocols (e.g. introduction of detailed mycotoxin testing) for cereal flours.

7.1.2 Pesticide residues in organic vs conventional flour – results from shopping basket study

More recently the intensive use of synthetic chemical pesticides (including herbicides, insecticides, nematicides and fungicides), although highly effective in controlling weeds, pests and diseases, have been linked to a range of negative side effects on human health from environmental and food-intake based exposure to pesticides including birth defects, reduced fertility, damage to the nervous system and cancer (Alavanja *et al.*, 2004; Rekha *et al.*, 2006; Aktar *et al.*, 2009; Nicolopoulou-Stamati *et al.*, 2016).

The two-year basket survey based on 297 samples indicated that (a) pesticide residues were found more than 5-times more frequently in conventional than organic flour samples and (b) that pesticide concentrations were up to 10-times higher in conventional wheat flour. Across the samples analysed there were five conventional white flour samples where pesticide levels reached or even exceeded the EU maximum residue limit allowed. This underlines the concern raised by consumers about nutritionally undesirable components especially pesticides which may be an advantage of consuming organic cereal products. Despite this, with a very small number of exceptions, the very low levels of the pesticide residues found in the flour samples examined, the long-term cumulative effect of consuming these contaminants has not been properly considered.

Due to insufficient published data so far, meta-analyses comparing pesticide residues between organic and conventional cereals are currently unreliable. However, as new data accumulate, this should be compared in future studies to gain a more complete understanding of the impacts of organic and conventional cereal management practises on the undesirable composition in cereals (and other plant crops).

7.2 Nutritional advantages, and its uncertainty of organic wheat – results from shopping basket study and field experiment (phenolic phytochemicals and nutritional minerals)

A range of epidemiological/cohort and dietary intervention studies carried out over the last two decades have reported positive associations between high dietary intakes of antioxidants-rich foods (whole grain cereals, fruit and vegetables) and a lower risk of cardiovascular disease, other oxidative stress related chronic/degenerative diseases and overall mortality (de Munter *et al.*, 2007; Pereira *et al.*, 2009; Gani *et al.*, 2012; Yang *et al.*, 2014; Williamson, 2017). In addition, micronutrient (e.g. Fe and Zn) deficiency affects more than 2-3 billion people worldwide (UNSCN, 2006). Micronutrient deficiency is common in developing countries, where staple cereals (wheat, maize or rice) provide most calories and diets are poor in meat, poultry, fish, fruits or vegetables (Bouis *et al.*, 2011). Screening studies have shown that modern wheat cultivars do not have a good gene pool to enhance the concentration of Zn and Fe (Zhao *et al.*, 2009b), probably because the breeding programs in which they were developed focused on maximizing yield rather than improving nutritional quality. Organic agronomic practices (rotation, organic manure and compost application) are thought to improve soil quality and micronutrient composition and concentration in cereals. Therefore, the demand for organic food and cereals is developing rapidly, which is thought to be partly due to consumers' expectations that organic food contains higher concentrations of nutritionally desirable phytochemicals (e.g. antioxidants/phenolics) and minerals.

The development of organic farming and the increasing demand for organic food triggered a wide range of studies to investigate the effects of agronomic management practices (organic and conventional) on the levels of phenolics/antioxidants and minerals in plant and animal-based organic foods. The recent meta-analysis based on 343 peer-review publications by Baranski *et al.* (2014) indicated statistically significant and meaningful differences in composition between organic and non-organic crops/crop based foods. Most importantly, the concentration of a range of antioxidants such as polyphenolics were found to be substantially higher in organic crops/crop-based foods, which was indicated by weighted meta-analysis. At the same time, slightly higher concentrations of Zn [5, (95% CI -6, 15)%] in organic crops were detected in the unweighted meta-analysis. The review by Worthington (2001b) also found that organic crops contained significantly more Fe, Mg and P than conventional crops.

The result of the SBS carried on in the thesis including 168 samples for phenolics/antioxidant and minerals from 2015-2016 found that, compared with conventional, organic common and spelt wheat flour had significantly higher concentrations of phenolics/antioxidants and health related minerals (Fe, Zn and P, Mg, S, K, Mn, Cu, Ni, Mo), which is consistent with the meta-

analysis by Baranski *et al.* (2014) and other previous studies (Worthington, 2001a; Helfenstein *et al.*, 2016).

However, the same limitations identified in the meta-analysis by Baranski *et al.* (2014), can be identified in the SBS, which included many uncertainties mainly related to both (a) the impact of organic production methods on composition and (b) potential impacts of organic food consumption on human health. It was impossible to access information for flour products tested on the specific agronomic management practice (soil management and tillage, crop rotation, fertilisation and crop protection regimes and variety choice) used to produce both organic and conventional common and Spelt wheat; this prevents linking phenolic/antioxidant concentration differences with specific management practices and confounding effects of choice of different varieties cannot be identified.

In the field experiment study carried on in this thesis, spelt wheat from the plots with organic fertilisation did not show the phenolic concentration advantages and concentration advantage of nutrition-related minerals including Zn compared with conventional fertilisation (only the concentration of Fe was significantly higher in organic manure fertilised spelt wheat), which was not consistent with the result of SBS and the meta-analysis by Baranski *et al.* (2014). This may be due to (a) the agronomic difference between organic and conventional farming systems examined which contribute to the composition may be not include the fertilisation treatment or (b) the confounding factors of climate, variety choice and agronomic practices or (c) the short period of the field experiment and (d) species choice. Therefore, recommendations should be developed for future farm surveys and long-term controlled experimental studies which would ensure that the impact of confounding factors is minimised (or at least recorded). Such information should include more details on management practices including agronomic practices such as rotation, impact of crop protection which are known to have potential effects on phenolics/antioxidant concentrations and mineral concentrations. Of course this information would still not be available for supermarket basket studies so the limitations for this type of studies would not be resolved.

7.3 Potential of organic agriculture

The main challenge of organic farming currently is to improve the yields close to or similar to those achieved in conventional farming systems. In the previous study by Bilsborrow *et al.* (2013), averaged across the four years, the conventional production system produced a wheat yield of 7.9 t/ha compared with 4.8 t/ha for the organic production system. This 40% reduction is consistent with many other studies comparing these productions systems for wheat where differences have been identified depending on site, year and management system used (Mäder *et al.*, 2002; Mason *et al.*, 2007; Jones *et al.*, 2010).

However, the field experiment study under semi-arid regions showed that organic fertiliser application produced similar, or even higher grain yields and lower lodging levels compared with mineral fertilisation. This may be due to the (a) use of a species and variety of Spelt which was well suited to this environment and to growth under organic conditions; (b) irrigation management; Spelt wheat is an ancient grain which has not gone through breeding with high input and pesticide use, therefore, it is hypothesized that spelt wheat has a higher nitrogen use efficiency for organic fertiliser (chicken and sheep manure) and is therefore ideal for low-input/organic systems. In addition, under semi-arid regions, although tillage — a non-production system-specific management parameter — had no significant effect on the concentration of minerals and phenolic/antioxidant, it can significant improve grain yield, which can be used in organic farming systems to improve the yield.

7.4 Nutritional quality of Wholemeal vs White flours

Currently the majority of wheat products are based on white flour (where the bran is removed and flour is made from the endosperm. However, the use of wholemeal flour (where the whole grain is milled), is increasingly recommend by nutritionists in recent decades because an increasing number of scientific studies have shown associations between whole grain consumption and a reduced risk of chronic disease such as cardiovascular disease, type 2 diabetes and obesity (Jones and Engleson, 2010; Cho *et al.*, 2013). The health benefits from whole grain consumption are thought to be mainly associated with the higher fibre, mineral and (poly)phenol/antioxidant content (which is mainly in the bran fraction of the grain) of wholemeal flour (Jones and Engleson, 2010). The SBS here confirmed that, compared with white flour, wholemeal common and spelt wheat flour contained consistently and significantly higher concentration (up to 2-3 times) of health beneficial phenolics/antioxidants and minerals.

However, there are also some studies which reported that the presence of higher concentrations of pesticides and mycotoxins in the bran fraction (which is removed when white flour is produced) (Weidenbörner *et al.*, 2000; Edwards *et al.*, 2011; Vidal *et al.*, 2013). In addition, concentrations of pesticide would also be expected to be higher in the outer layers/bran fraction of the grain (Bordin *et al.*, 2017). The SBS comparing mycotoxin and pesticide contamination between white and wholemeal flour is, to our knowledge, the first of its type, not considered in any investigation. The results of the retail survey study show that wholegrain flour and white flour has similar concentration of DON, ZEA and OTA, and though there is significant difference for T-2/HT-2, the concentration of T-2/HT-2 in wholegrain and white flour is more than 12 times lower than the MCL set by the EU. Therefore, there is no difference in mycotoxin-related health risks between white and wholegrain wheat consumption, and concerns about mycotoxin loads should not restrict nutritional recommendations to switch

from white to wholegrain consumption, given the increasing evidence for health benefits of wholegrain (McRae M. 2017). However, for the pesticide residues, conventional wholemeal flour contained more than 2-times higher pesticides residues than conventional white flour, which is consistent with several previous studies (Weidenbörner *et al.*, 2000; Edwards *et al.*, 2011; Vidal *et al.*, 2013). In contrast, pesticide residues concentrations in organic flour were not only substantially lower, but also similar in wholegrain and white flour.

Thus, there is a potential trade-off for the consumer between higher levels of compounds such as phenolics phytochemicals which have a potential benefit and higher levels of compounds such as pesticides which may be detrimental to health, however, it can be avoided through switching to organic wholemeal cereals and cereal based products.

7.5 Nutritional quality of spelt wheat vs common wheat

A recent study comparing the performance of traditional (grown until the 1960's) and modern common wheat varieties in an organic production system in the USA showed that mineral micronutrient concentrations (e.g. Se, Zn) were significantly lower in modern compared with traditional varieties (Murphy *et al.*, 2008), probably because the breeding programs in which they were developed focused on maximizing yield rather than improving nutritional quality (Guzmán *et al.*, 2014).

According to the three-year SBS, it was found that, compared with common wheat, spelt wheat had significantly higher concentration of phenolics/antioxidant, protein and several important nutrition-related minerals including Zn, K, and Mg. At the same time, spelt wheat had significantly lower DON and OTA but higher T-2 toxin concentrations. In terms of pesticides, it was shown spelt wheat had significant lower concentrations of chlormequat.

In addition, the field experiment study found that the concentration of total phenolics ranged from 9.5 µmol/g to 11.4 µmol/g and the minerals Fe and Zn ranged from 33.2 mg/kg, 29.5 mg/kg to 38.9 mg/kg, 41.2 mg/kg, respectively, between four varieties of ancient spelt wheat. This supports the previous study by Gomez-Becerra *et al.* (2010a) that identified various spelt varieties and pointed out that the potential health benefits and nutritional quality of minor cereals such as spelt wheat and the importance of variety selection and breeding.

7.6 Further work

To more comprehensively understanding of the nutritional composition difference of common wheat, spelt wheat and other cereals between organic and conventional production system, following further researches are required:

(a) A meta-analysis based on peer-review publications comparing difference of (i) phenolics/antioxidant concentration, (ii) nutritionally mineral and heavy metal concentration and (iii) pesticide residues; between organic and conventional cereals;

(b) The farm surveys which could collect detailed information of farm characteristics and management practices, pedo-climatic condition during the growing year, to better understand the effect of climate and agronomic practices on nutritional composition of cereals;

(c) Well controlled field experiment studies which ensure that the impact of confounding factors is minimised for mycotoxins to better understand the effect of specific agronomic practices (fertility treatment, crop rotation, pesticide application and others).

(d) Research focus on improving agronomic protocols and genetic resistance against mycotoxin producing *Fusarium* and mould fungi, especially in regions for which climate change is predicted to increase mycotoxin pressure.

Appendices

Appendix 1 Publications produced under the project

Wang, Juan, Marcin Barański, Carlo Leifert, Gavin B. Stewart, and Chris Seal. (2018) "The Protocol: Mycotoxin Content in Organically Versus Conventionally Cultivated Crops: A Systematic Literature Review and Meta-Analysis." OSF, https://osf.io/yc5eb/.

Wang, Juan, Lize Wood, P O Iverson, L Rempelos, Marcin Barański and Carlo Leifert (2019) 'Mycotoxin contamination of organic and conventional cereals and cereal-based products: result of the systematic literature review and meta-analysis '.

Wang, J., Wood, L., Anagnostopoulos, C., Ampadogiannis, G., Bempelou, E., Kiousi, M., Markelou, E., Bernhoft, A., Iverson, P.-O., Seal, C., Baranski, M., Vigar, V., Leifert, C. and Rempelos, L. (2019) 'Effect of harvest year, species (Triticum aestivum vs T. spelta), farming system (organic vs conventional) and flour type (wholegrain vs white) on mycotoxin concentrations in wheat flour – results of a retail survey in the UK and Germany '.

Wang, J., Wood, L., Anagnostopoulos, C., Ampadogiannis, G., Bempelou, E., Kiousi, M., Markelou, E., Iverson, P.-O., Seal, C., Baranski, M., Ellis, K., Vigar, V., Leifert, C. and Rempelo, L. (2019) 'Effect of harvest year, species (Triticum aestivum vs T. spelta), farming system (organic vs conventional) and flour type (wholegrain vs white) on pesticide concentrations in wheat flour – results of a retail survey in the UK and Germany',.

Appendix 2.1 List of information for extraction

Information about the study	▪ Study citation ▪ Year of publication ▪ Type of publishing (journal/conference article, report, poster, thesis, book chapter, unpublished data ▪ Peer review (yes, no) ▪ Geographic location of the study (country) ▪ Funding source ▪ Year of experiment
Experiment characteristics, that may acts as effect modifiers	▪ Comparator (management system short description, e.g. org, conv, int, etc.) ▪ Farm is certified or not ▪ The body certify farm as organic ▪ Cereal species (common wheat, barley, oat, spelt wheat, rye, rice, emmer, buckwheat, sorghum, millet, triticale, fonio, quinoa, other ▪ Type of participant (grain, flour, pasta, breakfast cereals, other) ▪ Climate (reference: World map of Koppen-Geiger climate classification) ▪ Rainfall ▪ Temperature ▪ Storage condition (temperature) and time (days) ▪ Analytical method for mycotoxin detection
Experiment details	▪ Sample moisture (%) ▪ Size of field (ha) ▪ Fertilisation type (mineral, farm yard/green manure, mixed) ▪ Fertilisation rate (N input, P input, K input) kg/ha ▪ Crop rotation type or sequence ▪ Preceding crop ▪ Soil type ▪ Soil moisture (%) ▪ The use of pesticide ▪ The use of fungicides ▪ The use of herbicides ▪ The use of insecticides ▪ The use of growth regulators ▪ Catch crops used ▪ Percentage of lodging ▪ Soil cultivation used (e.g. reduced tillage, harrowed, ploughed)
Outcome measurements (for each mycotoxin)	▪ Name of mycotoxin ▪ Number of participants (sample size) ▪ Contamination rate (% of positive samples) ▪ Concentration (mean or median value) ▪ Measure of variability (standard deviation or standard error) ▪ Minimum concentration in samples ▪ Maximum concentration in samples ▪ Measurement unit (name) ▪ Calculation basis (fresh or dry weight) ▪ Limit of detection (LOD) in the measurement unit ▪ Limit of quantification (LOQ)in the measurement unit ▪ Difference between comparators found by the author (no, sign, ns)
Other information	▪ Description of participants ('farms' in CF, 'replicates' in EX, 'samples' in BS) ▪ Data source in paper (table, figure, text)

Appendix 2.2 Risk of bias assessment

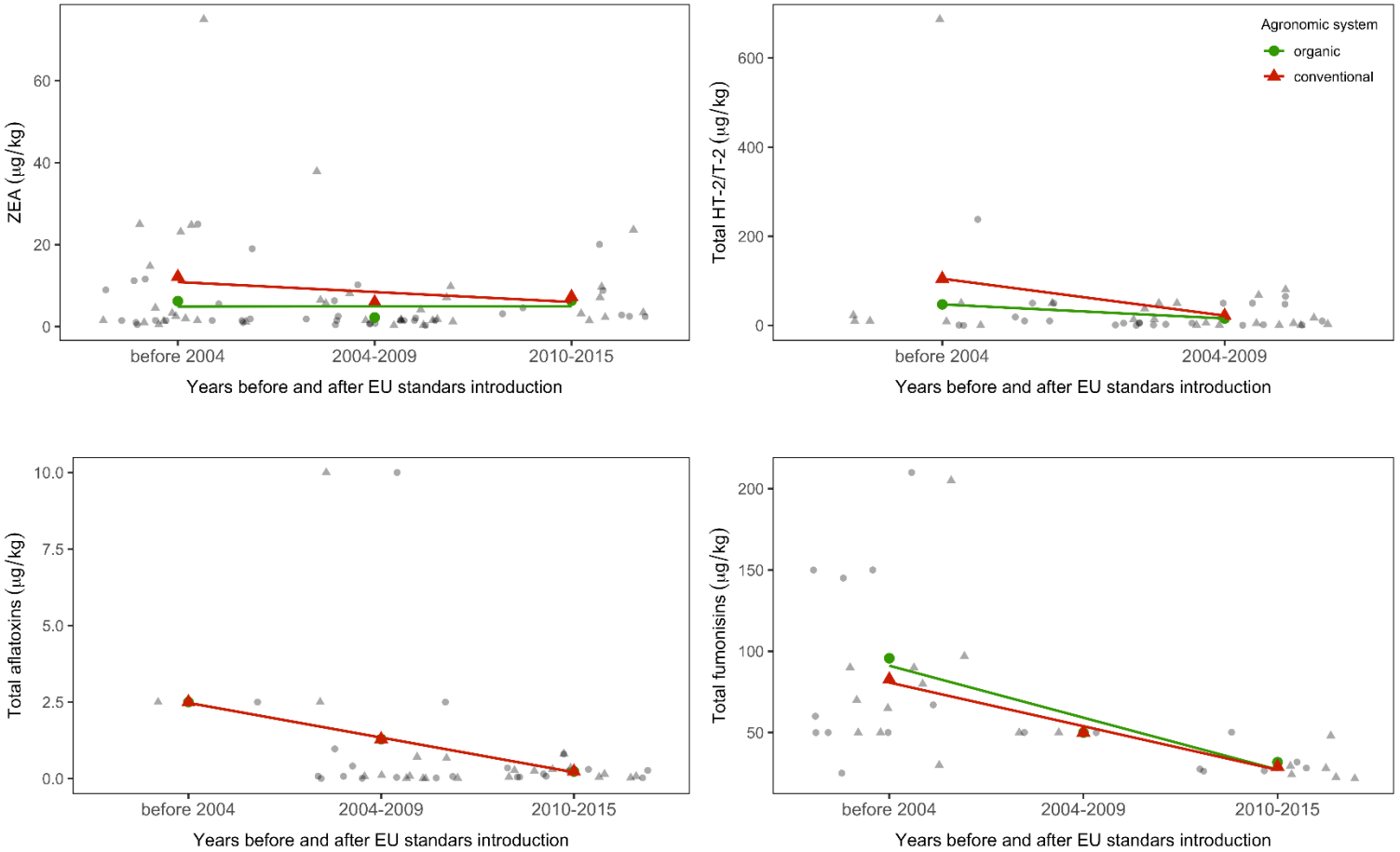
Item	Quality assessment statement
Study overview	▪ The study address agronomic question/hypothesis ▪ The type of study is clearly explained (field experiment, farm survey, shopping basket study)
Internal Validity	▪ Comparison is made between appropriate agronomic systems in terms of question/hypothesis ▪ The number of replicates (sample size) is described ▪ The number of replicates (sample size) is sufficient for statistical evaluation (>3,yes) ▪ The number of replicates (sample size) is the same or similar for all agronomic systems ▪ Each agronomic system are sufficiently described (CF, EX) / sampling places are described (BS) ▪ The geographic location of the experiment is the same for all agronomic systems ▪ The season and cultivation conditions (e.g. climate, soil properties) are the same or similar for all agronomic systems, except factors used to test question/hypothesis ▪ The variety of plants used in the study is the same for all agronomic systems
Analytical methods	▪ Samples selection is described ▪ Samples selection is the same for all agronomic systems ▪ The post-sampling storage time and conditions are described ▪ The post-sampling storage time and conditions are the same for all agronomic systems ▪ Choice of statistical methods is appropriate
Results	▪ Outcome measures are reliable and adequate to test question/hypothesis ▪ Effect sizes are given as mean or median values for each agronomic system ▪ The measurement of variance is provided for each mean (as confidence intervals, standard error, etc.) ▪ All outcome measures described in the methods section are reported (in tables, figures or text)
Overall assessment	▪ The limitation of the study design is discussed ▪ Authors discuss whether an effect found in study can be seen in the real life ▪ Study successfully minimise the risk of bias or confounding ▪ There is a clear evidence of an association between agronomic systems and outcome ▪ The sponsorship/conflict of interest is reported
Final rating	▪ ‘High quality’ when majority of criteria are met, there is a little or no risk of bias, and results are complete and well described ▪ ‘Acceptable’ when most criteria are met, there is low risk of bias, and results are complete and well described ▪ ‘Low quality’ when either most criteria are not met, or there is significant risk of bias relating to key aspects of study design and results are incomplete

Appendix 2.3 Citation, country (Ct), experiment year (ExpYear), study type (St), climate zone (Clim), analytical method (Met) and funding information of the publications included in the meta-analysis.

Citation	Ct*	ExpYear	St	Clim**	Met[&]	Funding
Alvito et al., 2010	PT	2007	BS	Csa	HPLC	not specified
Armorini et al., 2015	IT	2013	BS	Cfa	HPLC	not specified
Bakutis et al., 2006	LT	2003	CF	Dfb	ELISA	not specified
Baydan et al., 2016	TR	2012-2013	BS	Dsa	HPLC	public
Beretta et al., 2002	IT	2000	BS	Csa	HPLC	public
Bernhoft et al., 2010	NO	2002-2004	CF	Mix	GC	not specified
Bernhoft et al., 2012	NO	2002-2004	CF	Mix	GC	public
Biffi et al., 2004	IT	2001-2002	BS	Cwa	HPLC	public
Birzele et al.,2002	DE	1997	EX	Cfb	HPLC	public
Blajet-Kosicka, 2014	PL	2009-2012	BS	Dfb	HPLC	public
Brera et al., 2005	IT	2000-2001	CF	Cfa	HPLC	not specified
Champeil et al., 2004	FR	2000	EX	Cfb	GC	public
Cirillo et al., 2003	IT	2001	BS	Cas	GC	not specified
Czerwiecki et al., 2002	PL	1997	CF	Dfb	HPLC	public
Czerwiecki et al., 2002	PL	1998	CF	Dfb	HPLC	public
Doll et al., 2002	DE	1998	BS	Mix	ELISA	not specified
Edwards, 2009	GB	2002-2005	EX	Cfb	GC	public
Edwards, 2009	GB	2001-2005	EX	Cfb	HPLC	public
Eltun, 1996	NO	1991	EX	Dfc	HPLC	public
Fagnano et al.,2012	IT	2009	EX	Cwa	HPLC	public
Gonzalez et al., 2006	Mix	2004	BS	Mix	HPLC	public
Gonzalez-Osnaya et al., 2007	Mix	2003	BS	Mix	HPLC	public
Gottschalk et al., 2007	DE	2005	BS	Dfb	HPLC	public
Gourama, 2015	US	2009-2013	BS	Dfb	ELISA	public
Harcz et al., 2007	AT	2002-2005	CF	Cfb	HPLC	public
Hernandez-Martinez et al., 2010	ES	2008	BS	Cfb	HPLC	public
Herrera et al., 2009	ES	2007	BS	Cfb	HPLC	public
Hietaniemi et al., 2004	FI	1997	EX	Dfc	GC	public and private
Hoogenboom et al., 2008	NL	2003	CF	Cfb	HPLC	public
Hyun Ee Ok et al., 2011	KR	2009	BS	Dwa	GC	public
Jestoi et al., 2004	FI	2002	BS	Dfb	HPLC	not specified
Jorgensen et al., 2002	DK	1992	BS	Dfb	HPLC	not specified
Juan et al., 2008	Mix	2005	BS	Mix	HPLC	public
Kirchheim et al., 2002	DE	2001	BS	Cfb	HPLC	not specified
Kirincic et al., 2015	SI	2008-2012	BS	Mix	HPLC	public
Klinglmayr et al., 2010	AT	2008	BS	Dfb	HPLC	not specified
Konosonoka et al., 2015	LV	2011	EX	Dfb	ELISA	public
Kuzdralinski et al., 2013	PL	2006-2008	CF	Dfb	ELISA	not specified
Lacko-Bartosva et al., 2011	SK	2007	EX	Dfb	HPLC	public
Lauber et al., 2005	DE	2003	BS	Cfb	HPLC	not specified

Mader et al., 2007	CH	1998	EX	Dfb	HPLC	public
Malmauret et al., 2002	FR	2001	CF	Cfb	GC	public
Malysheva et al., 2014	mix	2012	BS	Mix	HPLC	public
Marx et al., 1995	DE	1991	CF	Dfb	HPLC	not specified
Mazurkiewicz et al., 2008	PL	2006	EX	Dfb	GC	public
Mazurkiewicz et al., 2008	PL	2006	EX	Dfb	GC	not specified
Meier et al., 2001	DE	1998	CF	Cfb	ELISA	public
Meister et al., 2004	DE	2000	CF	Dfb	HPLC	public
Meister et al., 2009	DE	2000	CF	Dfb	HPLC	public
Munger et al., 2014	CA	2009	EX	Dfb	ELISA	public
Nguyen et al., 2014	US	2012-2013	BS	Mix	HPLC	public
Ostrowska-Kolodziejczak et al., 2016	PL	2011	CF	Dfb	GC	not specified
Papouskova et al., 2015	CZ	2011-2012	EX	Dfb	HPLC	public
Perkowski et al., 2007	PL	2003	CF	Dfb	HPLC	not specified
Petr et al., 2009	CZ	1995	EX	Dfb	HPLC	public
Pleadin et al., 2017	HR	2015	BS	Cfa	ELISA	public
Pussemier et al., 2006	BE	2002	BS	Cfb	HPLC	public
Quaranta et al., 2010	IT	2006-2008	EX	Csa	ELISA	not specified
Reiter et al., 2010	AT	2008	BS	Mix	HPLC	public
Remza et al., 2016	SK	2009-2011	BS	Dfb	HPLC	public
Rodriguez et al., 2016	ES	2014	BS	BSk	GC	not specified
Rossi et al., 2006	IT	2004	CF	Cfa	HPLC	public
Schneweis et al., 2005	DE	1999	EX	Dfb	GC	public
Schollenberger et al., 1999	DE	1998	BS	Cfb	HPLC	not specified
Schollenberger et al., 2002	DE	1999	BS	Cfb	GC	not specified
Schollenberger et al., 2003	DE	1998-1999	BS	Cfb	GC	not specified
Schollenberger et al., 2005	DE	1999	BS	Cfb	GC	not specified
Serrano et al., 2013	ES	2011	BS	BSk	HPLC	public
Stanciu et al., 2017	RO	2014-2015	BS	Dfb	HPLC	public
Twaruzek et al., 2013	PL	2009-2011	BS	Dfb	HPLC	not specified
Vanova et al., 2008	CZ	2004	EX	Dfb	HPLC	public
Vidal et al., 2013	ES	2012	BS	BSk	HPLC	not specified
Vrcek et al., 2014	HR	2008	BS	Cfa	HPLC	public

*country codes according ISO 3166-2 published by the International Organization for Standardization; **climate zones according Köppen-Geiger climate classification (Peel *et al.*, 2007); CF, comparison of Farms; BS, shopping basket study; EX, Controlled Experiment; BSk: arid, steppe, cold weather; Csa: temperate weather with dry and hot summer; Cfa: code weather without dry season and with hot summer; Cfb: Temperate weather without dry season and with warm summer; Cwa: temperate weather with dry winter and with hot summer; Dfb: cold weather without dry season and with warm summer; Dfc: cold weather without dry season and with cold summer, Dsa: cold weather with dry summer and with hot summer; Dwa: cold weather with dry winter and with hot summer; Mix: more than one climate type;
***HPLC: high-performance liquid chromatography; GC: gas chromatography; ELISA: enzyme-linked immunosorbent assay.



Appendix 2.4 Concentration of *Fusarium* mycotoxins other than DON found in organic and conventional cereals and cereal products in comparative studies carried out between 1992-2015. First order regressions lines are between means of data from studies carried out before 2004, between 2004 and 2009, and/or between 2010 and 2015 and indicate changes in organic (green lines) and conventional (red lines). Grey circle and triangles represent individual data points for organic and conventional samples respectively from all studies included in the unweighted meta-analyses. A, zearalenone (ZEA), B, total HT-2/T-2 (included T-2, HT-2, T-2 tetraol and T-2 triol), and C, total aflatoxins (included AFB_1, AFB_2, AFG_1), D, total fumonisins (included FB_1, FB_2 and FUMS);

Appendix 4.1 Main effect means ± SE and *p*-values for the effects and interaction between wheat species, farming system and flour type on phenolic content of flour collected from UK and DE between 2015 and 2016

	Phenolics Content			
	Free	**Bound**	**Conjugated**	**Total**
Factor	µmol GAE g^{-1} flour (DW)			
Species				
Spelt (n=55)	4.9 ±0.2	3.9 ±0.4	0.7 ±0.1	9.4 ±0.4
Wheat (n=112)	4.6 ±0.1	3.4 ±0.3	0.6±0.1	8.5 ±0.4
farming system				
Conventional (n=84)	4.9 ±0.2	3.0 ±0.3	0.5 ±0.1	8.4 ±0.4
Organic (n=83)	4.4 ±0.1	4.1 ±0.3	0.7 ±0.1	9.2 ±0.4
Flour Type				
White (n=90)	4.5 ±0.1	1.2 ±0.1	0.36 ±0.1	6.0 ±0.2
Wholemeal (n=77)	4.9 ±0.2	6.3 ±0.2	0.89 ±0.1	12.1 ±0.3
ANOVA- results (p-values)				
Main Effects				
Species (SP)	**0.0176**	**0.0330**	**0.0386**	**0.0053**
Farming System (FS)	**0.0037**	**<0.0001**	**0.0001**	**0.0232**
Flour Type (FT)	**0.0024**	**<0.0001**	**<0.0001**	**<0.0001**
Interactions				
SP × FS	NS	NS	NS	NS
SP × FT	**0.0001** [1]	**0.0022** [1]	**0.0004** [1]	**<0.0001** [1]
FS × FT	NS	*0.0690*	NS	*0.0742*
SP × FS × FT	NS	NS	NS	NS

[1]See appendix 4.2 for Interaction means ± SE;

Appendix 4.2 Interactions means ± SE for the effects of wheat species and flour type on phenolic content in flour collected from UK and DE between 2015 and 2016.

Folin	**Factor 1**	**Factor 2** **flour type**	
Fraction	**wheat species**	White	Wholemeal
Free	spelt	5.1 ±0.3 **A a**	4.7 ±0.2 **A a**
µmol GAE g^{-1} flour (DW)	wheat	4.3 ±0.1 **B b**	5.1 ±0.3 **A a**
Bound	spelt	1.5 ±0.3 **B a**	5.8 ±0.3 **A b**
µmol GAE g^{-1} flour (DW)	wheat	1.1 ±0.1 **B a**	6.7 ±0.3 **A a**
Conjugated	spelt	0.5 ±0.1 **B b**	0.8 ±0.1 **A b**
µmol GAE g^{-1} flour (DW)	wheat	0.3 ±0.1 **B a**	0.9 ±0.1 **A a**
Total	spelt	7.1 ±0.4 **B a**	11.2 ±0.4 **A b**
µmol GAE g^{-1} flour (DW)	wheat	5.6 ±0.2 **B b**	12.7 ±0.4 **A a**

For each parameter assessed means labelled with the same lower case letter within the same row or column are not significant different (Turkey's honestly significant difference test $P<0.05$)

Appendix 4.3 Main effect means ± SE and *p*-values for the effects and interaction between wheat species, farming system and flour type on Antioxidant capacity by FRAP of flour collected from UK and DE between 2015 and 2016

	Antioxidant capacity by FRAP			
	Free	**Bound**	**Conjugated**	**Total**
	µmol $FeSO_4$ $7H_2O$ g^{-1} flour (DW)			
Species				
Spelt (n=55)	1.2 ±0.1	3.3 ±0.3	0.6 ±0.1	5.1 ±0.4
Wheat (n=112)	1.2 ±0.1	3.0 ±0.3	0.7 ±0.1	4.8 ±0.4
Farming system				
Conventional (n=84)	1.0 ±0.1	2.7 ±0.26	0.6 ±0.1	4.3 ±0.4
Organic (n=83)	1.4 ±0.1	3.6 ±0.29	0.7 ±0.1	5.5 ±0.4
Flour Type				
White (n=90)	0.7 ±0.1	1.0 ±0.2	0.4 ±0.1	2.0 ±0.1
Wholemeal (n=77)	1.7 ±0.1	5.6 ±0.3	1.0 ±0.1	8.3 ±0.2
ANOVA- results (p-values)				
Main Effects				
Species (SP)	NS	**0.0306**	NS	NS
Farming System (FS)	**0.0005**	**<0.0001**	NS	**<0.0001**
Flour Type (FT)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Interactions				
SP × FS	NS	NS	NS	NS
SP × FT	**0.0391** [1]	**0.0005** [1]	NS	**0.0002** [1]
FS × FT	**0.0256** [2]	**0.0379** [2]	NS	**0.0131** [2]
SP × FS × FT	NS	NS	NS	NS

[1]See appendix 4.4 for Interaction means ± SE; [2]See appendix 4.5 for Interaction means ± SE;

Appendix 4.4 Interactions means ± SE for the effects of wheat species and flour type on antioxidant capacity by FRAP of flour collected from UK and DE between 2015 and 2016.

FRAP	**Factor 1**	**Factor 2 flour type**	
Fraction	**wheat species**	White	Wholemeal
Free	spelt	0.7 ±0.1 **B a**	1.6 ±0.1 **A a**
µmol $FeSO_4\,7H_2O\ g^{-1}$ flour (DW)	wheat	0.7 ±0.1 **B a**	1.9 ±0.1 **A b**
Bound	spelt	1.1 ±0.2 **B a**	5.1 ±0.3 **A b**
µmol $FeSO_4\,7H_2O\ g^{-1}$ flour (DW)	wheat	0.9 ±0.1 **B a**	6.0 ±0.2 **A a**
Total	spelt	2.2 ±0.3 **B a**	7.4 ±0.3 **A b**
µmol $FeSO_4\,7H_2O\ g^{-1}$ flour (DW)	wheat	1.9 ±0.1 **B a**	8.9 ±0.3 **A a**

For each parameter assessed means labelled with the same lower case letter within the same row or column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.5 Interactions means ± SE for the effects of farming system and flour type on antioxidant capacity by FRAP of flour collected from UK and DE between 2015 and 2016

FRAP	**Factor 1**	**Factor 2 farming system**	
Fraction	**flour Type**	organic	conventional
Free	white	0.7 ±0.1 **A b**	0.7 ±0.1 **A b**
µmol $FeSO_4\,7H_2O\ g^{-1}$ flour (DW)	wholemeal	1.9 ±0.1 **A a**	1.5 ±0.1 **B a**
Bound	white	1.0 ±0.1 **A b**	1.0 ±0.1 **A b**
µmol $FeSO_4\,7H_2O\ g^{-1}$ flour (DW)	wholemeal	5.9 ±0.2 **A a**	5.3 ±0.2 **B a**
Total	white	2.0 ±0.2 **A b**	2.0 ±0.2 **A b**
µmol $FeSO_4\,7H_2O\ g^{-1}$ flour (DW)	wholemeal	8.7 ±0.3 **A a**	7.8 ±0.3 **B a**

For each parameter assessed means labelled with the same lower case letter within the same row or column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.6 Main effect means ± SE and *p*-values for the effects and interaction between wheat species, farming system and flour type on Antioxidant capacity by TEAC of flour collected from UK and DE between 2015 and 2016

	Antioxidant capacity by TEAC			
	Free	**Bound**	**Conjugated**	**Total**
	µmol Trolox g^{-1} flour (DW)			
Species				
Spelt (n=55)	2.0 ±0.2	6.5 ±0.6	1.2 ±0.2	9.8 ±0.8
Wheat (n=112)	1.9 ±0.1	5.6 ±0.5	1.0 ±0.2	8.5 ±0.6
Farming system				
Conventional (n=84)	1.8 ±0.1	5.1 ±0.5	0.9 ±0.2	7.8 ±0.6
Organic (n=83)	2.0 ±0.1	6.7 ±0.5	1.3 ±0.3	10.1 ±0.7
Flour Type				
White (n=90)	1.5 ±0.1	2.0 ±0.1	0.4 ±0.1	3.9 ±0.2
Wholemeal (n=77)	2.4 ±0.1	10.5 ±0.3	1.9 ±0.3	14.8 ±0.4
ANOVA- results (p-values)				
Main Effects				
Species (SP)	NS	**0.0152**	NS	**0.0380**
Farming System (FS)	NS	**<0.0001**	NS	**<0.0001**
Flour Type (FT)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Interactions				
SP × FS	NS	NS	NS	NS
SP × FT	NS	**0.0045** [1]	0.5488	**0.0112** [1]
FS × FT	*0.0824*	NS	NS	*0.0750*
SP × FS × FT	NS	NS	NS	NS

[1]See appendix 4.7 for Interaction means ± SE;

Appendix 4.7 Interactions means ± SE for the effects of wheat species and flour type on antioxidant capacity by TEAC of flour collected from UK and DE between 2015 and 2016

TEAC	**Factor 1**	**Factor 2** **flour Type**	
Fraction	**wheat species**	white	wholemeal
Bound	spelt	2.4 ±0.4 **B a**	9.6 ±0.6 **A b**
µmol Trolox g^{-1} flour (DW)	wheat	1.9 ±0.1 **B a**	11.0 ±0.4 **A a**
Total	spelt	4.5 ±0.5 **B a**	13.8 ±0.6 **A b**
µmol Trolox g^{-1} flour (DW)	wheat	3.7 ±0.2 **B a**	15.4 ±0.6 **A a**

For each parameter assessed means labelled with the same lower case letter within the same row or column are not significant different (Turkey's honestly significant difference test $P<0.05$)

Appendix 4.8 Main effect means ± SE and *p*-values for the effects and interaction between wheat species, farming system and flour type on flavonoid content in flour collected from UK and DE between 2015 and 2016

	Flavonoid Content			
	Free	**Bound**	**Conjugated**	**Total**
	µmol Catechin g^{-1} flour (DW)			
Species				
Spelt (n=55)	0.2±0.1	0.6±0.1	0.1 ±0.03	1.0 ±0.1
Wheat (n=112)	0.3±0.1	0.6±0.1	0.1 ±0.02	1.0 ±0.1
Farming system				
Conventional (n=84)	0.2±0.1	0.5±0.1	0.1 ±0.01	0.8 ±0.1
Organic (n=83)	0.3±0.1	0.7±0.1	0.2 ±0.03	1.1 ±0.1
Flour Type				
White (n=90)	0.3±0.05	0.2±0.03	0.1 ±0.02	0.6 ±0.1
Wholemeal (n=77)	0.2±0.01	1.0±0.06	0.2 ±0.02	1.4 ±0.1
ANOVA- results (p-values)				
Main Effects				
Species (SP)	NS	NS	NS	NS
Farming System (FS)	NS	*0.0704*	*0.0566*	**0.0094**
Flour Type (FT)	NS	**<0.0001**	**0.0011**	**<0.0001**
Interactions				
SP × FS	NS	NS	NS	NS
SP × FT	NS	NS	NS	NS
FS × FT	NS	NS	**0.0022** [1]	NS
SP × FS × FT	NS	*0.0697*	NS	**0.0198** [2]

[1]See appendix 4.9 for Interaction means ± SE; [2]See appendix 4.10 for the interaction means ± SE.

Appendix 4.9 Interactions means ± SE for the effects of farming system and flour type on antioxidant capacity by flavonoid in flour collected from UK and DE between 2015 and 2016

Flavonoid	**Factor 1**	**Factor 2**	
		farming system	
Fraction	**flour type**	organic	conventional
conjugated	white	0.1 ±0.1 **A a**	0.1 ±0.1 **A a**
µmol Catechin g^{-1} flour (DW)	wholemeal	0.2 ±0.1 **A a**	0.1 ±0.1 **A a**

For each parameter assessed means labelled with the same lower case letter within the same row or column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.10 Interactions means ± SE for the effects of wheat species, farming system and flour type on flavonoid content in bound fraction of flour collected from UK and DE between 2015 and 2016

Flavonoid	**Factor 1**	**Factor 2**	**Factor 3**	
			farming system	
Fraction	**wheat species**	**flour type**	organic	conventional
Total	spelt	white	0.5 ±0.1 **A b**	0.6 ±0.1 **A b**
		wholemeal	1.5 ±0.1 **A a**	1.1 ±0.1 **A a**
µmol Catechin g^{-1} flour (DW)	wheat	White	0.8 ±0.2 **A b**	0.5 ±0.1 **B b**
		wholemeal	1.5 ±0.1 **A a**	1.5 ±0.2 **A a**

For each parameter assessed means labelled with the same lower case letter within the same row are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.11 Main effect means ± SE and *p*-values for the effects and interaction between wheat species, farming system and flour type on ferulic acid detected by HPLC in flour collected from UK and DE between 2015 and 2016

	Ferulic Acid (HPLC)			
	Free	**Bound**	**Conjugated**	**Total**
	µmol/g flour(DW)			
Species				
Spelt (n=55)	0.8 ±0.1	355.2 ±33.0	13.5 ±0.9	369.5 ±33.7
Wheat (n=112)	0.9 ±0.1	338.9 ±29.4	12.5 ±0.7	352.3 ±30.1
Farming system				
Conventional (n=84)	0.8 ±0.1	322.2 ±33.7	10.9 ±0.7	333.8 ±34.3
Organic (n=83)	0.9 ±0.1	366.6 ±29.7	14.8 ±0.9	382.3 ±30.5
Flour Type				
White (n=90)	0.5 ±0.1	112.6 ± 8.1	7.2±0.4	120.3 ± 8.4
Wholemeal (n=77)	1.3 ±0.1	615.1 ±22.7	19.4±0.6	635.7 ±22.8
ANOVA- results (p-values)				
Main Effects				
Species (SP)	**0.0164**	NS	NS	NS
Farming System (FS)	*0.0855*	NS	**<.0001**	**0.0887**
Flour Type (FT)	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Interactions				
SP × FS	NS	NS	NS	NS
SP × FT	*0.0861*	**0.0002** [1]	*0.0613*	**0.0002** [1]
FS × FT	NS	NS	**0.0034** [2]	NS
SP × FS × FT	NS	**0.0052** [3]	NS	**0.0062** [3]

[1]See table appendix 4.12 for Interaction means ± SE; [2]See table appendix 4.13 for Interaction means ± SE; [3]See table appendix 4.14 for Interaction means ± SE

Appendix 4.12 Interactions means ± SE for the effects of wheat species and flour type on ferulic phenolic acid concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016

	Factor 1	**Factor 2** **flour type**	
Fraction	**wheat species**	white	wholemeal
		Ferulic Acid (HPLC)	
Bound	spelt	122.8 ±23.6 **B a**	535.2 ±25.9 **A a**
µmol g^{-1} flour (DW)	wheat	108.9 ± 6.9 **B a**	668.9 ±31.5 **A b**
Total	spelt	130.9 ±24.2 **B a**	554.2 ±26.2 **A b**
µmol g^{-1} flour (DW)	wheat	116.5 ± 7.4 **B a**	690.6 ±31.5 **A a**

For each parameter assessed means labelled with the same lower case letter within the same row or column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.13 Interactions means ± SE for the effects of farming system and flour type on ferulic acid concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016

	Factor 1	**Factor 2** **flour type**	
Fraction	**farming system**	white	wholemeal
		Ferulic Acid (HPLC)	
conjugated	conventional	6.9 ±0.6 **B a**	17.0 ±0.7 **A b**
µmol g^{-1} flour (DW)	organic	7.6 ±0.5 **B a**	21.1 ±0.8 **A a**

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.14 Interactions means ± SE for the effects of wheat species, flour type and farming system on ferulic phenolic acid concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016

	Factor 1	**Factor 2**	**Factor 3 flour type**	
Fraction	**species**	**farming system**	White	Wholemeal
			Ferulic Acid (HPLC)	
Bound	spelt	conventional	150.6 ±44.2 **B a**	492.4 ±57.0 **A c**
		organic	94.9 ±15.2 **B a**	558.7 ±25.0 **A bc**
µmol g^{-1} flour (DW)	wheat	conventional	105.0 ± 8.9 **B a**	715.7 ±52.2 **A a**
		organic	114.5 ±11.4 **B a**	626.0 ±36.0 **A b**
Total	spelt	conventional	159.1 ±45.4 **B a**	509.4 ±57.8 **A c**
		organic	102.7 ±15.7 **B a**	578.9 ±25.0 **A bc**
µmol g^{-1} flour (DW)	wheat	conventional	112.1 ± 9.4 **B a**	734.6 ±52.3 **A a**
		organic	122.7 ±12.0 **B a**	650.4 ±36.0 **A b**

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.15 Main effect means ± SE and *p*-values for the effects and interaction between wheat species, farming system and flour type on Sinapic acids detected by HPLC in flour collected from UK and DE between 2015 and 2016

	Sinapic Acid (HPLC)			
	Free	**Bound**	**Conjugated**	**Total**
	µmol g^{-1} flour (DW)			
Species				
Spelt (n=55)	5.5 ±0.7	8.0 ±0.9	14.8 ±1.4	28.3 ±2.6
Wheat (n=112)	4.3 ±0.5	9.4 ±1.0	14.9 ±1.1	28.6 ±2.4
Farming system				
Conventional (n=84)	3.7 ±0.5	8.9 ±1.2	13.4 ±1.2	26.0 ±2.6
Organic (n=83)	5.7 ±0.6	9.0 ±0.9	16.4 ±1.3	31.1 ±2.5
Flour Type				
White (n=90)	2.8 ±0.4	2.0 ±0.3	6.8 ±0.7	11.6 ±1.2
Wholemeal (n=77)	6.9 ±0.7	17.1 ±0.9	24.3 ±1.0	48.3 ±1.9
ANOVA- results (p-values)				
Main Effects				
Species (SP)	**0.0432**	NS	NS	NS
Farming System (FS)	**0.0029**	NS	**0.0138**	**0.0299**
Flour Type (FT)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Interactions				
SP × FS	NS	NS	NS	NS
SP × FT	0.0331	**<0.0001** [1]	**0.0011** [1]	**<0.0001** [1]
FS × FT	NS	*0.0903*	*0.0612*	NS
SP × FS × FT	NS	**0.0166** [2]	NS	NS

[1]See appendix 4.16 for Interaction means ± SE; [2]See appendix 4.17 for Interaction means ± SE;

Appendix 4.16 Interactions means ± SE for the effects of wheat species and flour type on sinapic acid concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016

	Factor 1	**Factor 2** **flour type**	
Fraction	**wheat species**	white	wholemeal
		Sinapic Acid (HPLC)	
Free	spelt	3.9 ±0.8 **B a**	6.8 ±1.0 **A a**
µmol g^{-1} flour (DW)	wheat	2.4 ±0.4 **B b**	7.0 ±0.9 **A a**
Conjugated	spelt	7.6 ±2.0 **B a**	20.4 ±1.3 **A b**
µmol g^{-1} flour (DW)	wheat	6.5 ±0.6 **B a**	26.9 ±1.2 **A a**
Total	spelt	14.0 ±3.3 **B a**	39.4 ±2.4 **A b**
µmol g^{-1} flour (DW)	wheat	10.7 ±1.1 **B a**	54.3 ±2.5 **A a**

For each parameter assessed means labelled with the same lower case letter within the same row or column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.17 Interactions means ± SE for the effects of wheat species, flour type and farming system on sinapic acid concentration in flour collected from UK and DE between 2015 and 2016

	Factor 1	**Factor 2**	**Factor 3** **flour type**	
Fraction	**wheat species**	**farming system**	white	wholemeal
			Sinapic Acid (HPLC)	
Bound	spelt	conventional	3.6 ±1.8 **B a**	12.4 ±1.7 **A c**
		organic	1.6 ±0.4 **B a**	12.1 ±0.9 **A c**
µmol g^{-1} flour (DW)	wheat	conventional	1.7 ±0.2 **B a**	22.9 ±1.9 **A a**
		organic	1.9 ±0.3 **B a**	18.0 ±1.3 **A b**

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.18 Main effect means ± SE and *p*-values for the effects and interaction between wheat species, farming system and flour type on 4-hydroxybenzoic acid concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016.

	4-hydroxybenzoic acid (HPLC)			
	Free	**Bound**	**Conjugated**	**Total**
Factor	µmol/g flour(DW)			
Species				
Spelt (n=55)	0.98 ±0.05	2.12 ±0.19	2.34 ±0.15	5.45 ±0.36
Wheat (n=112)	1.08 ±0.06	1.95 ±0.16	1.98 ±0.10	5.01 ±0.30
Farming system				
Conventional (n=84)	1.03 ±0.06	1.90 ±0.19	1.83 ±0.10	4.76 ±0.33
Organic (n=83)	1.07 ±0.06	2.11 ±0.17	2.37 ±0.13	5.55 ±0.33
Flour Type				
White (n=90)	0.73 ±0.04	0.70 ±0.06	1.30 ±0.06	2.73 ±0.13
Wholemeal (n=77)	1.42 ±0.05	3.53 ±0.12	3.03 ±0.09	7.98 ±0.19
ANOVA- results (p-values)				
Main Effects				
Species (SP)	NS	NS	**0.0025**	**0.0444**
Production System (FS)	NS	NS	**<.0001**	**0.0014**
Flour Type (FT)	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Interactions				
SP × FS	NS	NS	NS	NS
SP × FT	**<.0001** [1]	**0.0001**	NS	**0.0001**
FS × FT	NS	NS	**0.0269** [2]	NS
SP × FS × FT	NS	**0.0104** [3]	NS	**0.0376** [3]

[1]See appendix 4.19 for Interaction means ± SE; [2]See appendix 4.20 for Interaction means ± SE; [3]See appendix 4.21 for Interaction means ± SE

Appendix 4.19 Interactions means ± SE for the effects of wheat species and flour type on 4-hydroxybenzoic acid concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016.

	Factor 1	**Factor 2**	
		Flour Type	
Fraction	**wheat species**	white	wholemeal
		4-hydroxybenzoic acid (HPLC)	
Free	spelt	0.76 ±0.07 **B a**	1.15 ±0.06 **A b**
µmol/g flour (DW)	wheat	0.71 ±0.05 **B a**	1.61 ±0.07 **A a**

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.20 Interactions means ± SE for the effects of farming system and flour type on 4-hydroxybenzoic acid concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016.

	Factor 1	**Factor 2**	
		Flour Type	
Fraction	**Farming system**	white	wholemeal
		4-hydroxybenzoic acid (HPLC)	
Conjugated	conventional	1.25 ±0.08 **B a**	2.72 ±0.09 **A b**
µmol/g flour (DW)	organic	1.38 ±0.07 **B a**	3.26 ±0.13 **A a**

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.21 Interactions means ± SE for the effects of wheat species, flour type and farming system on 4-hydroxybenzoic acid concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016.

	Factor 1	**Factor 2**	**Factor 3 Flour Type**	
Fraction	**wheat species**	**farming system**	white	wholemeal
			4-hydroxybenzoic acid (HPLC)	
Bound	spelt	conventional	1.09 ±0.36 **B a**	2.93 ±0.36 **A c**
		organic	0.67 ±0.08 **B a**	3.18 ±0.17 **A bc**
µmol/g flour (DW)	wheat	conventional	0.62 ±0.04 **B b**	4.10 ±0.25 **A a**
		organic	0.66 ±0.06 **B a**	3.59 ±0.19 **A b**
Total	spelt	conventional	3.44 ±0.65 **B a**	6.72 ±0.56 **A b**
		organic	2.81 ±0.21 **B ab**	7.54 ±0.36 **A b**
µmol/g flour (DW)	wheat	conventional	2.51 ±0.17 **B b**	8.48 ±0.30 **A a**
		organic	2.71 ±0.21 **B ab**	8.47 ±0.31 **A a**

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4. 22 Main effect means ± SE and *p*-values for the effects and interaction between wheat species, farming system and flour type on vanillic acid detected by HPLC in flour collected from UK and DE between 2015 and 2016

	vanillic acid (HPLC)			
	Free	**Bound**	**Conjugated**	**Total**
	µmol/g flour(DW)			
Species				
Spelt (n=55)	0.75 ±0.07	2.18 ±0.19	3.15 ±0.18	6.08 ±0.41
Wheat (n=112)	0.77±0.06	2.28 ±0.20	2.80 ±0.17	5.85 ±0.41
Farming system				
Conventional (n=84)	0.73 ±0.07	2.13 ±0.22	2.60 ±0.17	5.47 ±0.44
Organic (n=83)	0.80 ±0.06	2.36 ±0.19	3.23 ±0.19	6.39 ±0.42
Flour Type				
White (n=90)	0.33 ±0.02	0.73 ±0.05	1.68 ±0.09	2.74 ±0.15
Wholemeal (n=77)	1.27 ±0.05	4.03 ±0.14	4.36 ±0.12	9.65 ±0.26
ANOVA- results (p-values)				
Main Effects				
Species (SP)	NS	NS	**0.0329**	NS
Farming System (FS)	NS	*0.0929*	**0.0003**	**0.0021**
Flour Type (FT)	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Interactions				
SP × FS	NS	NS	NS	NS
SP × FT	**0.0002** [1]	**<0.0001**	**0.0010** [1]	**<.0001** [1]
FS × FT	NS	NS	NS	NS
SP × FS × FT	NS	**0.0240** [2]	NS	NS

[1]See appendix 4.23 for Interaction means ± SE; [2]See appendix 4.24 for Interaction means ± SE;

Appendix 4.23 Interactions means ± SE for the effects of wheat species and flour type on vanillic acid concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016.

	Factor 1	**Factor 2**	
		flour Type	
Fraction	**wheat species**	White	Wholemeal
		vanillic acid (HPLC)	
Free	spelt	0.35 ±0.04 **B a**	1.06 ±0.07 **A b**
µmol/g flour (DW)	wheat	0.33 ±0.02 **B a**	1.41 ±0.07 **A a**
Conjugated	spelt	2.03 ±0.19 **B a**	4.01 ±0.16 **A b**
µmol/g flour (DW)	wheat	1.55 ±0.10 **B b**	4.59 ±0.17 **A a**
Total	spelt	3.24 ±0.36 **B a**	8.28 ±0.29 **A b**
µmol/g flour (DW)	wheat	2.56 ±0.16 **B a**	10.58 ±0.32 **A a**

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.24 Interactions means ± SE for the effects of wheat species, flour type and farming system on vanillic acid concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016.

	Factor 1	**Factor 2**	**Factor 3**	
			flour type	
Fraction	**wheat Species**	**farming system**	White	Wholemeal
			vanillic acid (HPLC)	
Bound	spelt	conventional	1.02 ±0.30 **B a**	3.09 ±0.34 **A c**
		organic	0.70 ±0.10 **B a**	3.27 ±0.13 **A c**
µmol/g flour (DW)	wheat	conventional	0.65 ±0.06 **B a**	4.89 ±0.26 **A a**
		organic	0.71 ±0.08 **B a**	4.29 ±0.23 **A b**

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.25 Main effect means ± SE and *p*-values for the effects and interaction between wheat species, production system and flour type on syringic acid detected by HPLC in flour collected from UK and DE between 2015 and 2016

	syringic acid (HPLC)			
	Free	**Bound**	**Conjugated**	**Total**
	µmol/g flour(DW)			
Species				
Spelt (n=55)	0.49 ±0.04	2.48 ±0.24	3.34 ±0.24	6.30 ±0.47
Wheat (n=112)	0.53 ±0.04	2.96 ±0.28	3.15 ±0.20	6.64 ±0.49
Farming system				
Conventional (n=84)	0.45 ±0.04	2.86 ±0.33	2.89 ±0.22	6.20 ±0.55
Organic (n=83)	0.59 ±0.04	2.75 ±0.25	3.54 ±0.22	6.87 ±0.48
Flour Type				
White (n=90)	0.30 ±0.02	0.78 ±0.06	1.71 ±0.09	2.79 ±0.16
Wholemeal (n=77)	0.77 ±0.04	5.18 ±0.25	4.96 ±0.18	10.91 ±0.36
ANOVA- results (p-values)				
Main Effects				
Species (SP)	NS	**0.0463**	NS	NS
Farming System (FS)	**0.0028**	NS	**0.0013**	**0.0197**
Flour Type (FT)	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Interactions				
SP × FS	NS	NS	NS	NS
SP × FT	**0.0007** [1]	**<.0001**	**0.0054** [1]	**<.0001**
FS × FT	NS	NS	NS	NS
SP × FS × FT	NS	**0.0026** [2]	NS	**0.0094** [2]

[1]See appendix 4.26 for Interaction means ± SE; [2]See appendix 4.27 for Interaction means ± SE;

Appendix 4.26 Interactions means ± SE for the effects of wheat species and flour type on syringic acid concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016.

	Factor 1	**Factor 2 flour Type**	
Fraction	**wheat species**	white	wholemeal
		syringic acid (HPLC)	
Free	spelt	0.31 ±0.03 **B a**	0.62 ±0.05 **A b**
µmol/g flour (DW)	wheat	0.30 ±0.03 **B a**	0.87 ±0.06 **A a**
Conjugated	spelt	1.89 ±0.19 **B a**	4.45 ±0.25 **A b**
µmol/g flour (DW)	wheat	1.64 ±0.10 **B a**	5.30 ±0.23 **A a**

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.27 Interactions means ± SE for the effects of wheat species, flour type and farming system on syringic acid concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016.

	Factor 1	**Factor 2**	**Factor 3 flour Type**	
Fraction	**wheat species**	**farming system**	**white**	**wholemeal**
			syringic acid (HPLC)	
Bound	spelt	conventional	1.10 ±0.35 **B a**	3.76 ±0.50 **A c**
		organic	0.54 ±0.06 **B a**	3.79 ±0.18 **A c**
µmol/g flour (DW)	wheat	conventional	0.76 ±0.06 **B a**	7.11 ±0.48 **A a**
		organic	0.77 ±0.09 **B a**	5.22 ±0.35 **A b**
Total	spelt	conventional	3.52 ±0.73 **B a**	8.42 ±0.82 **A c**
		organic	2.51 ±0.19 **B a**	9.10 ±0.32 **A c**
µmol/g flour (DW)	wheat	conventional	2.56 ±0.20 **B a**	13.00 ±0.72 **A a**
		organic	2.92 ±0.29 **B a**	11.65 ±0.54 **A b**

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.28 Main effect means ± SE and *p*-values for the effects and interaction between wheat species, farming system and flour type on p-coumaric acid concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016.

	p-coumaric acid (HPLC)			
	Free	**Bound**	**Conjugated**	**Total**
	µmol/g flour(DW)			
Species				
Spelt (n=55)	2.73 ±0.33	12.53 ±1.23	1.34 ±0.12	16.60 ±1.49
Wheat (n=112)	2.54 ±0.27	10.17 ±0.96	1.60 ±0.15	14.31 ±1.25
Farming system				
Conventional (n=84)	2.15 ±0.26	9.12 ±0.97	0.98 ±0.09	12.25 ±1.20
Organic (n=83)	3.06 ±0.33	12.80 ±1.14	2.06 ±0.19	17.92 ±1.48
Flour Type				
White (n=90)	1.56 ±0.19	3.70 ±0.32	0.84 ±0.09	6.10 ±0.49
Wholemeal (n=77)	3.83 ±0.35	19.41 ±0.92	2.30 ±0.18	25.55 ±1.22
ANOVA- results (p-values)				
Main Effects				
Species (SP)	NS	**0.0227**	NS	**0.079**
Farming System (FS)	**0.0066**	**0.0005**	**<.0001**	**<.0001**
Flour Type (FT)	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Interactions				
SP × FS	NS	*0.0579*	**0.0027** [1]	**0.0406** [1]
SP × FT	**0.0061** [2]	*0.0815*	NS	**0.0163** [2]
FS × FT	NS	*0.0598*	**0.0055** [3]	**0.0278** [3]
SP × FS × FT	NS	NS	NS	NS

[1]See appendix 4.29 for Interaction means ± SE; [2]See appendix 4.30 for Interaction means ± SE; [3]See appendix 4.31 for Interaction means ± SE

Appendix 4.29 Interactions means ± SE for the effects of wheat specie and production system on p-coumaric acid concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016.

	Factor 1	Factor 2 farming system	
Fraction	**wheat species**	conventional	organic
		p-coumaric acid (HPLC)	
Conjugated	spelt	1.15 ±0.20 **A a**	1.48 ±0.15 **A b**
µmol/g flour (DW)	wheat	0.91 ±0.09 **B a**	2.42 ±0.28 **A a**
Total	spelt	15.27±2.56 **A a**	17.56 ±1.81 **A a**
µmol/g flour (DW)	wheat	11.11±1.33 **B a**	18.14 ±2.13 **A a**

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.30 Interactions means ± SE for the effects of wheat specie and flour type on p-coumaric acid concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016.

	Factor 1	Factor 2 flour type	
Fraction	**wheat species**	white	wholemeal
		p-coumaric acid (HPLC)	
Free	spelt	1.93 ±0.37 **B a**	3.35 ±0.49 **A a**
µmol/g flour (DW)	wheat	1.42 ±0.21 **B a**	4.15 ±0.49 **A a**
Total	spelt	7.51 ±1.24 **B a**	23.65 ±1.56 **A a**
µmol/g flour (DW)	wheat	5.59 ±0.48 **B a**	26.83 ±1.74 **A a**

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.31 Interactions means ± SE for the effects of production system and flour type on p-coumaric acid concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016.

	Factor 1	Factor 2 flour type	
Fraction	**farming system**	white	wholemeal
		p-coumaric acid (HPLC)	
Conjugated	conventional	0.58 ±0.05 **B b**	1.59 ±0.16 **A b**
µmol/g flour (DW)	organic	1.17 ±0.18 **B a**	2.84 ±0.26 **A a**
Total	conventional	5.38 ±0.67 **B a**	22.86 ±1.64 **A b**
µmol/g flour (DW)	organic	7.04 ±0.69 **B a**	27.56 ±1.69 **A a**

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.32 Main effect means ± SE and *p*-values for the effects and interaction between wheat species, production system and flour type on syringaldeyde concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016

	syringaldeyde			
	Free	**Bound**	**Conjugated**	**Total**
	µmol/g flour(DW)			
Species				
Spelt (n=55)	0.19 ±0.02	4.16 ±0.41	1.47 ±0.12	5.81 ±0.52
Wheat (n=112)	0.41 ±0.07	4.28 ±0.40	1.31 ±0.09	6.00 ±0.50
Farming system				
Conventional (n=84)	0.49 ±0.10	3.88 ±0.43	1.10 ±0.09	5.47±0.55
Organic (n=83)	0.19 ±0.02	4.60 ±0.41	1.62 ±0.11	6.41±0.51
Flour Type				
White (n=90)	0.22 ±0.03	1.29±0.14	0.67 ±0.06	2.18 ±0.19
Wholemeal (n=77)	0.48 ±0.10	7.69 ±0.32	2.16 ±0.08	10.32 ±0.39
ANOVA- results (p-values)				
Main Effects				
Species (SP)	*0.0568*	NS	*0.0786*	NS
Farming System (FS)	**0.0080**	**0.0499**	**<.0001**	**0.0302**
Flour Type (FT)	**0.0004**	**<.0001**	**<.0001**	**<.0001**
Interactions				
SP × FS	*0.0552*	NS	*0.0973*	NS
SP × FT	*0.0835*	**<.0001** [1]	NS	**<.0001** [1]
FS × FT	*0.0777*	NS	**0.0433** [2]	NS
SP × FS × FT	NS	NS	NS	NS

[1]See table appendix 4.33 for Interaction means ± SE; [2]See appendix 4.34 for Interaction means ± SE;

Appendix 4.33 Interactions means ± SE for the effects of wheat species and flour type on syringaldeyde concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016.

	Factor 1	**Factor 2 flour Type**	
Fraction	**wheat species**	White	Wholemeal
		syringaldeyde (HPLC)	
Bound	spelt	1.70 ±0.42 **B a**	6.05 ±0.38 **A b**
µmol/g flour (DW)	wheat	1.14 ±0.11 **B a**	8.79 ±0.41 **A a**
Total	spelt	2.55 ±0.55 **B a**	8.34 ±0.44 **A b**
µmol/g flour (DW)	wheat	2.05 ±0.16 **B a**	11.67 ±0.49 **A a**

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.34 Interactions means ± SE for the effects of farming system and flour type on syringaldeyde concentration detected by HPLC in flour collected from UK and DE between 2015 and 2016.

	Factor 1	**Factor 2 flour type**	
Fraction	**farming system**	White	Wholemeal
		syringaldeyde (HPLC)	
Conjugated	conventional	0.59 ±0.07 **B a**	1.89 ±0.1 **A b**
µmol/g flour (DW)	organic	0.79 ±0.09 **B a**	2.36 ±0.1 **A a**

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.35 Main effect means ± SE and *p*-values for the effects and interaction between wheat species, farming system and flour type on total concentration of phenolic acids detected by HPLC in flour collected from UK and DE between 2015 and 2016

	Total phenolic components (HPCL)			
	Free	**Bound**	**Conjugated**	**Total**
	µmol/g flour(DW)			
Species				
Spelt (n=55)	11.69±1.07	386.78±35.9	39.96±3.01	438.43±39.07
Wheat (n=112)	10.82±0.88	370.02±32.2	38.32±2.44	419.15±34.98
Farming system				
Conventional (n=84)	9.61±0.86	351.11±36.79	33.71±2.44	394.43±39.55
Organic (n=83)	12.61±1.05	400.27±32.49	44.06±2.84	456.95±35.73
Flour Type				
White (n=90)	6.51±0.59	121.83±8.91	20.3±1.32	148.65±10.44
Wholemeal (n=77)	16.47±1.03	672.09±24.54	60.54±1.84	749.10±25.90
ANOVA- results (p-values)				
Main Effects				
Species (SP)	NS	NS	NS	NS
Farming System (FS)	**0.0096**	NS	**<.0001**	*0.0502*
Flour Type (FT)	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Interactions				
SP × FS	NS	NS	*0.0926*	NS
SP × FT	**0.0012** [1]	**0.0001**	**0.0036** [1]	**0.0001**
FS × FT	NS	NS	**0.0152** [2]	NS
SP × FS × FT	NS	**0.0066** [3]	NS	**0.0109** [3]

[1]See appendix 4.36 for Interaction means ± SE; [2]See appendix 4.37 for Interaction means ± SE; [3]See appendix 4.38 for Interaction means ± SE

Appendix 4.36 Interactions means ± SE for the effects of wheat species and flour type on total concentration of phenolic acids detected by HPLC in flour collected from UK and DE between 2015 and 2016

Fraction	Factor 1 wheat species	Factor 2 flour type	
		white	wholemeal
		Total phenolic components (HPCL)	
Free	spelt	7.9 ±1.2 **B a**	14.6 ±1.5 **A a**
µmol/g flour (DW)	wheat	6.0 ±0.7 **B a**	17.7 ±1.4 **A a**
Conjugated	spelt	22.0 ±3.4 **B a**	53.9 ±2.7 **A b**
µmol/g flour (DW)	wheat	19.7 ±1.3 **B a**	65.0 ±2.2 **A a**

For each parameter assessed means labelled with the same lower case letter within the same row or column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.37 Interactions means ± SE for the effects of farming system and flour type on total concentration of phenolic acids detected by HPLC in flour collected from UK and DE between 2015 and 2016

Fraction	Factor 1 farming system	Factor 2 flour Type	
		white	wholemeal
		Total phenolic components (HPCL)	
Conjugated	conventional	19.6 ±2.0 **B a**	55.5 ±2.2 **A b**
µmol/g flour (DW)	organic	21.2 ±1.5 **B a**	64.3 ±2.6 **A a**

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.38 Interactions means ± SE for the effects of wheat species, flour type and farming system on total concentration of phenolic acids detected by HPCL in flour collected from UK and DE between 2015 and 2016.

Fraction	Factor 1 wheat species	Factor 2 farming system	Factor 3 flour Type	
			white	wholemeal
			Total phenolic components (HPCL)	
Bound	Spelt	conventional	165.2 ±49.6 **B a**	539.2 ±62.6 **A c**
		organic	104.0 ±16.9 **B a**	605.6 ±26.8 **A bc**
µmol/g flour (DW)	Wheat	conventional	112.7 ± 9.6 **B a**	781.1 ±56.2 **A a**
		organic	123.7 ±12.4 **B a**	688.5 ±38.3 **A b**
Total	Spelt	conventional	196.6 ±56.4 **B a**	603.3 ±65.9 **A b**
		organic	132.4 ±19.6 **B a**	676.5 ±29.4 **A b**
µmol/g flour (DW)	Wheat	conventional	136.2 ±11.9 **B a**	855.6 ±58.3 **A a**
		organic	152.5 ±14.8 **B a**	778.8 ±41.3 **A a**

For each parameter assessed means labelled with the same capital letter with row and same lower case letter within the column are not significant different (Turkey's honestly significant difference test P<0.05)

Appendix 4.39 Correlation coefficients between total phenolic content (TPC) and antioxidant capacity and between different measure of antioxidant capacity

Fraction	PC vs FRAP	PC vs TEAC	PC vs FLA	FRAP vs TEAC	FRAP vs FLA	TEAC vs FLA
free	0.312**	-0.037	0.057	0.686**	0.082	0.067
bound	0.960**	0.853**	0.646**	0.934**	0.704**	0.721**
conjugated	0.404**	0.278**	0.148	0.059	0.048	-0.006
total	0.883**	0.700**	0.504**	0.859**	0.621**	0.543**

*,** were significant at 0.05, 0.01 probability level, respectively

Appendix 4.40 Main effect means ± SE and p-values for the effects of, and interactions between year (2016 and 2017), country (Germany VS UK), farming system (organic vs conventional) and flour type (white vs wholegrain) on mycotoxin concentrations in **common wheat flour** samples.

	Mycotoxin concentration (µg/kg)			
Factor	**DON***	**T-2/HT-2***	**ZEA***	**OTA***
Year				
2016 (n=77)	36± 9	2.1±0.3	6.8±0.5	3.6±0.2
2017 (n=134)	67±12	0.5±0.2	2.5±0.2	2.7±0.1
Country				
Germany (n=93)	44± 9	0.9±0.2	4.3±0.4	2.2±0.1
UK (n=118)	66±13	1.2±0.2	3.8±0.3	3.7±0.2
Farming System				
Conventional (n=120)	52± 8	1.1±0.2	3.9±0.3	2.9±0.1
Organic (n=91)	62±16	1.0±0.2	4.2±0.4	3.2±0.2
Flour Type				
White (n=144)	48± 9	0.6±0.1	3.5±0.3	3.0±0.1
Wholegrain (n=67)	74±16	2.2±0.4	5.1±0.5	3.0±0.2
Maximum residue level (µg/kg) (EC 2006&2013)	750	50	75	3
ANOVA results (p-values)				
Main Effects				
Year (YR)	NS	**<0.0001**	**<0.0001**	**0.0022**
Country (CT)	NS	NS	**0.0153**	**<0.0001**
Farming System (FS)	*0.0978*	NS	NS	**0.0489**
Flour Type (FT)	*0.092*	**<0.0001**	**0.0381**	NS
Interactions				
YR × CT	*0.0616*	**0.0181**	*0.0738*	**0.0295**
YR × FS	NS	NS	NS	**0.0256**
CT × FS	**0.0197**	NS	NS	*0.0703*
YR × FT	NS	**0.0001**	NS	**0.039**
CT × FT	NS	**0.0022**	NS	**0.0425**
FS × FT	NS	**0.0447**	NS	NS
YR × CT × FS	*0.0831*	NS	NS	**0.0131**
YR × CT × FT	NS	NS	NS	NS
YR × FS × FT	NS	NS	**0.0034**	**0.0188**
SP × FS × FT	**0.0019**	NS	NS	*0.0874*
YR × CT × FS × FT	NS	NS	NS	NS

* p-values are after log(x+1) transformation;

Appendix 4.41 Main effect means ± SE and p-values for the effects of, and interactions between, year (2015 VS 2017), country (Germany vs UK), farming system (organic vs conventional) and flour type (white vs wholegrain) on mycotoxin concentrations in **spelt wheat flour** samples.

	Mycotoxin concentration (µg/kg)			
Factor	**DON***	**T-2/HT-2***	**ZEA**	**OTA***
Year				
2015 (n=20)	53± 8	1.5±0.4	1.9±0.3	3.0±0.3
2017 (n=50)	42± 9	0.4±0.1	1.5±0.2	2.5±0.2
Country				
Germany (n=45)	49±10	0.8±0.2	1.6±0.2	2.3±0.2
UK (n=25)	38± 8	0.6±0.3	1.7±0.3	3.4±0.2
Farming System				
Conventional (n=26)	67±14	0.6±0.3	1.8±0.3	2.8±0.2
Organic (n=44)	32± 7	0.8±0.2	1.5±0.2	2.6±0.2
Flour Type				
White (n=36)	40±10	0.7±0.2	1.6±0.2	2.4±0.2
Wholegrain (n=34)	50±10	0.8±0.2	1.7±0.2	2.9±0.2
Maximum residue level (µg/kg) (EC 2006&2013)	750	50	75	3
ANOVA results (p-values)				
Main Effects				
Year (YR)	NS	*NS*	NS	NS
Country (CT)	NS	NS	NS	**0.0029**
Farming System (FS)	**0.0086**	NS	NS	NS
Flour Type (FT)	NS	NS	NS	NS
Interactions				
YR × CT	NS	NS	NS	NS
YR × FS	NS	NS	NS	NS
CT × FS	NS	NS	NS	NS
YR × FT	NS	*0.0797*	NS	NS
CT × FT	NS	NS	NS	NS
FS × FT	NS	*0.0622*	*0.096*	NS
YR × CT × FS	NS	NS	NS	*0.0513*
YR × CT × FT	NS	NS	NS	NS
YR × FS × FT	NS	NS	NS	NS
SP × FS × FT	NS	NS	NS	NS
YR × CT × FS × FT	NS	**0.0061**	NS	NS

*P-values for DON, T-2/HT-2 and OTA are after log(x+1) transformation.

Appendix 4.42 List of pesticide analysed in both 2016 and 2017 and their limits of detection (LOQ).

Pesticides assessed in both years (2016 and 2017)	**LOQ* (mg/kg)**		No. of positive samples		Fungicide/Herbicides	Chemical Group	Approved by EU or not
	2016	**2017**	**2016**	**2017**			
2-phenylphenol	0.01	0.01	-	**12**	Fungicide	Phenol	Approved. Not a permitted food additive, allowed as a post-harvest treatment (only in 4 EU countries)
Acetamiprid	0.01	0.01	-	-	Insecticide	chloropyridinyl neonicotinoids	Approved
Azoxystrobin	0.01	0.01	-	-	Fungicide	Strobilurin	Approved
Bifenthrin	0.01	0.01	-	-	Insecticide, Acaricide	Pyrethroid	Approved. Only uses as insecticide in greenhouses with a permanent structure may be authorised
Boscalid	0.10	0.01	-	-	Fungicide	Carboxamide	Approved
Carbendazim	0.01	0.01	-	-	Fungicide	Benzimidazole	Not
Chlormequat	0.01	0.01	**25**	**66**	Plant growth regulator	Quarternary ammonium compound	Approved
Chlorpropham	0.02	0.01	-	-	Herbicide, Plant growth regulator	Carbamate	Approved
Chlorpyrifos	0.01	0.01	-	-	Insecticide, Acaricide	Organophosphate	Approved
Chlorpyrifos-methyl	0.01	0.01	-	**1**	Insecticide, Acaricide	Organophosphate	Approved
Clothianidin	0.01	0.01	-	-	Insecticide	Neonicotinoid	Not
Cyfluthrin	0.01	0.01	-	-	Insecticide, Acaricide	Pyrethroid	Not
Cypermethrin	0.01	0.01	**15**	**-**	Insecticide, Acaricide	Pyrethroid	Approved
Cyproconazole	0.01	0.01	-	-	Fungicide	Triazole	Approved
Cyprodinil	0.01	0.01	-	-	Fungicide	Anilinopyrimidine	Approved
Deltamethrin	0.01	0.01	**5**	**5**	Insecticide	Pyrethroid	Approved

Diazinon	0.01	0.01	-	-	Insecticide, Acaricide	Organophosphate	Not
Difenoconazole	0.01	0.01	-	-	Fungicide	Triazole	Approved
Dimethoate	0.01	0.01	-	-	Insecticide, Acaricide	Organophosphate	Approved
Fenhexamid	0.01	0.01	-	-	Fungicide	Fenhexamid	Approved
Fenpropimorph	0.01	0.01	-	-	Fungicide	Morpholine	Approved
Fenvalerate	0.01	0.01	-	-	Insecticide, Acaricide	Pyrazolium	Not
Folpet	0.01	0.01	-	-	Fungicide	Phthalimide	Approved
Glyphosate	**3.00**	**0.01**	-	**22**	Herbicide	Phosphonoglycine	Approved
Imazalil	0.01	0.01	-	-	Fungicide	Imidazole	Approved
Imidacloprid	0.01	0.01	-	-	Insecticide	Neonicotinoid	Approved
Metalaxyl	0.01	0.01	-	-	Fungicide	Phenylamide	Approved
Metribuzin	0.02	0.01	-	-	Herbicide	Triazinone	Approved
Omethoate	0.01	0.01	-	-	Insecticide, Acaricide	Organophosphate	Not
Pendimethalin	0.01	0.01	-	-	Herbicide	Dinitroaniline	Approved
Piperonyl butoxide	0.01	0.01	**31**	**22**	Not a plant protection product	Cyclic aromatic	NA
Pirimicarb	0.01	0.01	-	-	Insecticide	Carbamate	Approved
Pirimicarb-desmethyl	0.01	0.01	-	-	NA		NA
Pirimiphos-ethyl	0.01	0.01	-	-	Insecticide	Organophosphate	Not
Pirimiphos-methyl	0.01	0.01	**3**	**10**	Insecticide	Organophosphate	Approved
Propiconazole	0.01	0.01	-	-	Fungicide	Triazole	Not
Pyraclostrobin	0.01	0.01	-	-	Fungicide, Plant growth regulator	Strobilurin	Approved
Pyrethrins	0.01	0.01	-	-	Insecticide	Plant derived	Approved
Pyrimethanil	0.01	0.01	-	-	Fungicide	Anilinopyrimidine	Approved
Spiroxamine	0.01	0.01	-	-	Fungicide	Morpholine	Approved

Tau-Fluvalinate	0.01	0.01	-	-	Insecticide	Synthetic pyrethroid	Approved
Tebuconazole	0.01	0.01	-	-	Fungicide	Triazole	Approved
Tefluthrin	0.02	0.01	-	-	Insecticide	Pyrethroid	Approved
Thiacloprid	0.01	0.01	-	-	Insecticide	Neonicotinoid	Approved
Thiamethoxam	0.01	0.01	-	-	Insecticide	Neonicotinoid	Approved
Thiophanate-methyl	0.01	0.01	-	-	Fungicide	Benzimidazole	Approved
Triadimenol	0.01	0.01	-	-	Fungicide	Triazole	Approved

*LOQ is the lowest concentration at which the analyse can not only be reliably detected but at which some predefined goals for bias and imprecision are met.

Total CPP residues include Deltamethrin, Chlormequat, Piperonyl butoxide, Pirimiphos-methyl and 2-phenylphenol; Chlorpyrifos-methyl and Glyphosate were excluded from the multiples CPP residues because Chlorpyrifos-methyl only has one positive sample and the LOQs for Glyphosate in 2016 and 2017 were different.

Appendix 4.43 Pesticide list analysed in 2016 and LOQs

Pesticide 2016	**LOQ (mg/kg)**	No. of positive samples	Fungicide/Herbicides	Chemical Group	Approved by EU or not
2,4-D	0.01	-	Herbicide, Plant growth regulator		Approved
2-phenylphenol	0.01	-	Fungicide	Phenol	Approved. Not a permitted food additive, allowed as a post-harvest treatment (only in 4 EU countries)
Acetamiprid	0.01	-	Insecticide	chloropyridinyl neonicotinoids	Approved
Azoxystrobin	0.01	-	Fungicide	Strobilurin	Approved
Bifenthrin	0.01	-	Insecticide, Acaricide	Pyrethroid	Approved. Only uses as insecticide in greenhouses with a permanent structure may be authorised
Boscalid	0.10	-	Fungicide	Carboxamide	Approved
Carbendazim	0.01	-	Fungicide	Benzimidazole	Not
Chlormequat	0.01	**25**	Plant growth regulator	Quarternary ammonium compound	Approved
Chlorpropham	0.02	-	Herbicide, Plant growth regulator	Carbamate	Approved
Chlorpyrifos	0.01	-	Insecticide, Acaricide	Organophosphate	Approved
Chlorpyrifos-methyl	0.01	-	Insecticide, Acaricide	Organophosphate	Approved
Clothianidin	0.01	-	Insecticide	Neonicotinoid	Not
Cyfluthrin	0.01	-	Insecticide, Acaricide	Pyrethroid	Not
Cyhalothrin-lambda	0.01	-	Insecticide	Pyrethroid	Not
Cypermethrin	0.01	**15**	Insecticide, Acaricide	Pyrethroid	Approved
Cyproconazole	0.01	-	Fungicide	Triazole	Approved
Cyprodinil	0.01	-	Fungicide	Anilinopyrimidine	Approved

DDT	0.01	-	Insecticide	Organochlorine	Not
Deltamethrin	0.01	**5**	Insecticide	Pyrethroid	Approved
Diazinon	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Difenoconazole	0.01	-	Fungicide	Triazole	Approved
Dimethoate	0.01	-	Insecticide, Acaricide	Organophosphate	Approved
Epoxiconazole	0.01	-	Fungicide	Triazole	Approved
Fenhexamid	0.01	-	Fungicide	Fenhexamid	Approved
Fenpropimorph	0.01	-	Fungicide	Morpholine	Approved
Fenvalerate	0.01	-	Insecticide, Acaricide	Pyrazolium	Not
Fluazifop (free acid)	0.05	-	Herbicide	Unclassified	Not
Fludioxonil	0.01	-	Fungicide	Phenylpyrrole	Approved
Flutriafol	0.01	-	Fungicide	Triazole	Approved
Folpet	0.01	-	Fungicide	Phthalimide	Approved
Glyphosate	3.00	-	Herbicide	Phosphonoglycine	Approved
Imazalil	0.01	-	Fungicide	Imidazole	Approved
Imidacloprid	0.01	-	Insecticide	Neonicotinoid	Approved
Malathion	0.01	-	Insecticide, Acaricide	Organophosphate	Approved
Mepiquat	0.01	**3**	Plant growth regulator	Quarternary ammonium compound	Approved
Metalaxyl	0.01	-	Fungicide	Phenylamide	Approved
Metribuzin	0.02	-	Herbicide	Triazinone	Approved
Omethoate	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Pendimethalin	0.01	**3**	Herbicide	Dinitroaniline	Approved
Piperonyl butoxide	0.01	**31**	Not a plant protection product	Cyclic aromatic	NA
Pirimicarb	0.01	-	Insecticide	Carbamate	Approved

Pirimicarb-desmethyl	0.01	-	NA		NA
Pirimiphos-ethyl	0.01	-	Insecticide	Organophosphate	Not
Pirimiphos-methyl	0.01	**3**	Insecticide	Organophosphate	Approved
Propiconazole	0.01	-	Fungicide	Triazole	Not
Pyraclostrobin	0.01	-	Fungicide, Plant growth regulator	Strobilurin	Approved
Pyrethrins	0.01	-	Insecticide	Plant derived	Approved
Pyrimethanil	0.01	-	Fungicide	Anilinopyrimidine	Approved
Spinosad	0.01	-	Insecticide	Micro-organism derived	Approved
Spiroxamine	0.01	-	Fungicide	Morpholine	Approved
Tau-Fluvalinate	0.01	-	Insecticide	Synthetic pyrethroid	Approved
Tebuconazole	0.01	**2**	Fungicide	Triazole	Approved
Tefluthrin	0.02	-	Insecticide	Pyrethroid	Approved
Thiacloprid	0.01	-	Insecticide	Neonicotinoid	Approved
Thiamethoxam	0.01	-	Insecticide	Neonicotinoid	Approved
Thiophanate-methyl	0.01	-	Fungicide	Benzimidazole	Approved
Triadimenol	0.01	-	Fungicide	Triazole	Approved

*LOQ is the lowest concentration at which the analyse can not only be reliably detected but at which some predefined goals for bias and imprecision are met

Appendix 4.44 *Pesticide list analysed in 2017 and LOQs.*

Pesticide List 2017	**LOQ (mg/kg)**	No. of positive samples	Fungicide/Herbicides	Chemical Group	Approved by EU or not
1,4-Dimethylnapthalene	0.01	-	Plant growth regulator		Not
2-(1-Naphthyl)acetamide	0.01	-			
2,4,6-Trichlorophenol	0.01	-	Fungicide, Herbicide	Unclassified	Not
2-Methyl-4,6-dinitrophenol	0.01	-			
2-Phenylphenol	0.01	**12**	Fungicide	Phenol	Approved. Not a permitted food additive, allowed as a post harvest treatment (only in 4 EU countries)
3-hydroxycarbofuran	0.01	-			
4,4-Dichlorobenzophenone	0.01	-			
6-Benzyladenine	0.01	-	Plant growth regulator		Approved
9,10-Anthraquinone	0.01	-	Repellent	Unclassified	Not
Abamectin	0.01	-	Insecticide, Acaricide		Approved
Acephate	0.01	-	Insecticide	organophosphate	Not
Acetamiprid	0.01	-	Insecticide	chloropyridinyl neonicotinoids	Approved
Acetochlor	0.01	-	Herbicide	Chloroacetamide	Not
Acibenzolar-S-methyl	0.01	-	Plant activator	Benzothiadiazole	Approved
Aclonifen	0.01	-	Herbicide	Diphenyl ether	Approved
Acrinathrin	0.01	-	Acaricide	Pyrethroid	Approved
Alachlor	0.01	-	Herbicide	Chloroacetamide	Not
Aldicarb	0.01	-	Insecticide, Acaricide, Nematicide	Carbamate	Not
Aldicarb sulphone	0.01	-			
Aldicarb sulphoxide	0.01	-			
Aldrin	0.01	-	NA	Organochlorine	Not

Ametryn	0.01	-	Herbicide	Triazine	Not
Aminocarb	0.01	-	Insecticide	Carbamate	Not
Amitraz	0.01	-	Insecticide, Acaricide	Amidine	Not
Atraton	0.01	-	Herbicide	Methoxytriazine	Not
Atrazine	0.01	-	Herbicide	Triazine	Not
Azaconazole	0.01	-	Fungicide, Insecticide	Triazole	Not
Azadirachtin	0.01	-	Insecticide		Approved
Azinphos ethyl	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Azinphos methyl	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Azobenzene	0.01	-	Acaricide, Ovicide, Miticide	Bridged diphenyl	Not
Azoxystrobin	0.01	-	Fungicide	Strobilurin	Approved
Benalaxyl	0.01	-	Fungicide	Acylamino acid	Approved
Bendiocarb	0.01	-	Insecticide	Carbamate	Not
Benfluralin	0.01	-	Herbicide	Dinitroaniline	Approved
Benfuracarb	0.01	-	Insecticide, Nematicide	Carbamate	Not
Benthiavalicarb-isopropyl	0.01	-			
Bifenazate	0.01	-	Insecticide, Acaricide	Hydrazine carboxylate	Approved
Bifenox	0.01	-	Herbicide	Diphenyl ether	Approved
Bifenthrin	0.01	-	Insecticide, Acaricide	Pyrethroid	Approved. Only uses as insecticide in greenhouses with a permanent structure may be authorised
Binapacryl	0.01	-	Fungicide, Insecticide, Miticide	Dinitrophenol	Not
Biphenyl	**0.05**	-	Fungicide	Aromatic hydrocarbon	Not
Bitertanol	0.01	-	Fungicide	Triazole	Not
Boscalid	0.01	-	Fungicide	Carboxamide	Approved
Bromacil	0.01	-	Herbicide	Uracil	Not
Bromophos	0.01	-	Insecticide	Organophosphate	Not

Bromophos-Ethyl	0.01	-	Insecticide	Organophosphate	Not
Bromopropylate	0.01	-	Acaricide	Benzilate	Not
Bromuconazole	0.01	-	Fungicide	Triazole	Approved
Bupirimate	0.01	-	Fungicide	Pyrimidinol	Approved
Buprofezine	0.01	-	Insecticide, Acaricide	Unclassified	Not
Butachlor	0.01	-	Herbicide	Chloroacetamide	Not
Butocarboxim	0.01	-	Insecticide	Butocarboxim	Not
Butoxycarboxim	0.01	-	Insecticide, Acaricide	Carbamate	Not
Butralin	0.01	-	Herbicide, Plant growth regulator	Dinitroaniline	Not
Cadusafos	0.01	-	Insecticide, Nematicide	Organophosphate	Not
Captan	0.01	-	Fungicide	Phthalimide	Approved
Carbaryl	0.01	-	Insecticide, Plant growth regulator	Carbamate	Not
Carbendazim	0.01	-	Fungicide	Benzimidazole	Not
Carbetamide	0.01	-	Herbicide	Carbamate	Approved
Carbofuran	0.01	-	Insecticide, Acaricide, Nematicide	Carbamate	Not
Carbophenothion	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Carboxine	0.01	-	Fungicide	Oxathiin	Not
Carfentrazone Ethyl	0.01	-	Herbicide		Approved
Carpropamid	0.01	-	Fungicide	Cyclopropanecarboxamide	Not
Chinomethionat	0.01	-	Fungicide, Acaricide	Carbamate	Not
Chlorantraniliprole	0.01	-	Insecticide	Anthranilic diamide	Approved
Chlorbenzilate	0.01	-			
Chlorbromuron	0.01	-	Herbicide	Urea	Not
Chlorbufam	0.01	-	Herbicide	Carbanilate	Not
Chlordane	0.01	-	NA	Organochlorine	Not, banned
Chlordimeform	0.01	-	Insecticide, Acaricide	Formamidine	Not
Chlorethoxyfos	0.01	-	Insecticide	Organophosphate	Not
Chlorfenapyr	0.01	-	Insecticide, Acaricide	Pyrrole	Not
Chlorfenson	0.01	-	Insecticide, Acaricide	Bridged diphenyl	Not
Chlorfenvinphos	0.01	-	Insecticide	Organophosphate	Not

Chlorfluazuron	0.01	-	Insecticide	Benzoylurea	Not
Chloridazon	0.01	-	Herbicide	Pyridazinone	Not
Chlormephos	0.01	-	Insecticide	Organophosphate	Not
Chlormequat	0.01	**66**	Plant growth regulator	Quarternary ammonium compound	Approved
Chloropropylate	0.01	-	Acaricide	Bridged diphenyl	Not
Chlorothalonil	0.01	-	Fungicide	Chloronitrile	Approved
Chlorotoluron	0.01	-	Herbicide	Urea	Approved
Chlorpropham	0.01	-	Herbicide, Plant growth regulator	Carbamate	Approved
Chlorpyrifos	0.01	-	Insecticide, Acaricide	Organophosphate	Approved
Chlorpyrifos methyl	0.01	**1**	Insecticide, Acaricide	Organophosphate	Approved
Chlorthal Dimethyl	0.01	-	Herbicide		Not
Chlorthion	0.01	-	Insecticide	Organophosphate	Not
Chlorthiophos	0.01	-	Insecticide	Organophosphate	Not
Chlozolinate	0.01	-	Fungicide	Oxazolidin	Not
cis-1,2,3,6-Tetrahydrophthalimide	0.01	-	Metabolite	Unclassified	Not
Clodinafop propargy	0.01	-	Herbicide	Aryloxyphenoxypropionate	Not
Clofentezine	0.01	-	Acaricide	Tetrazine	Approved
Clomazone	0.01	-	Herbicide	Isoxazolidinone	Approved
Cloquintocet mexyl	0.01	-	Not a plant protection product	Unclassified	NA
Clothianidin	0.01	-	Insecticide	Neonicotinoid	Not
Coumaphos	0.01	-	Insecticide		Not
Cyanazine	0.01	-	Herbicide	Triazine	Not
Cyazofamid	0.01	-	Fungicide	Cyanoimidazole	Approved
Cycluron	0.01	-	Herbicide	Urea	Not
Cyflufenamid	0.01	-	Fungicide	Amidoxine	Approved
Cyfluthrin	0.01	-	Insecticide, Acaricide	Pyrethroid	Not
Cymoxanil	0.01	-	Fungicide	Cyanoacetamide oxime	Approved
Cypermethrin	0.01	-	Insecticide, Acaricide	Pyrethroid	Approved

Cyproconazole	0.01	-	Fungicide	Triazole	Approved
Cyprodinil	0.01	-	Fungicide	Anilinopyrimidine	Approved
Cyromazine	0.01	-	Insecticide	Triazine	Approved
Cythioate	0.01	-			
DEET	0.01	-			
Deltamethrin	0.01	**5**	Insecticide	Pyrethroid	Approved
Demeton	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Demeton-s-methyl	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Demeton-s-methyl sulphone	0.01	-	Insecticide	Organophosphate	Not
Desmedipham	0.01	-	Herbicide	Carbamate	Approved
Desmetryn	0.01	-	Herbicide	Methylthiotriazine	Not
Diafenthiuron	0.01	-	Insecticide, Acaricide	Thiourea	Not
Dialifos	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Diazinon	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Dichlobenil	0.01	-	Herbicide	Benzonitrile	Not
Dichlofenthion	0.01	-	Insecticide	Organophosphate	Not
Dichlofluanid	0.01	-	Fungicide	Sulphamide	Not
Dichlorvos	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Diclobutrazol	0.01	-	Fungicide	Conazole	Not
Dicloran	0.01	-	Fungicide	Chlorophenyl	Not
Dicofol	0.01	-	Acaricide	Organochlorine	Not
Dicrotophos	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Dieldrin	0.01	-	Insecticide	Chlorinated hydrocarbon	Not, banned
Diethofencarb	0.01	-	Fungicide	Carbamate	Approved
Difenoconazole	0.01	-	Fungicide	Triazole	Approved
Diflubenzuron	0.01	-	Insecticide	Benzoylurea	Approved
Diflufenican	0.01	-	Herbicide	Carboxamide	Approved
Dimefuron	0.01	-	Herbicide	Oxadiazolone/phenylurea	Not
Dimethenamid	0.01	-	Herbicide	Chloroacetamide	Not

Dimethoate	0.01	-	Insecticide, Acaricide	Organophosphate	Approved
Dimethomorph	0.01	-	Fungicide	Morpholine	Approved
Dimoxystrobin	0.01	-	Fungicide	Strobilurin	Approved
Diniconazole	0.01	-	Fungicide	Triazole	Not
Dinotefuran	0.01	-	Insecticide	Neonicotinoid	Not
Dinoterb	0.01	-	Herbicide	Dinitrophenol	Not
Dioxabenzofos	0.01	-	Insecticide	Organophosphate	Not
Dioxacarb	0.01	-	Insecticide	Carbamate	Not
Diphenamid	0.01	-	Herbicide	Alkanamide	Not
Diphenylamine	0.01	-	Plant growth regulator	Amine	Not
Disulfoton	0.01	-	Insecticide	Organophosphate	Not
Disulfoton sulfoxide	0.01	-	Metabolite	Organophosphate	
Disulfoton sulphone	0.01	-			
Ditalimfos	0.01	-	Fungicide	Organophosphate	Not
Diuron	0.01	-	Herbicide	Phenylamide	Approved
DMSA	0.01	-			
DMST	0.01	-			
Dodemorph	0.01	-	Fungicide	Morpholine	Approved
Dodine	0.01	-	Fungicide	Guanidine	Approved
Edifenphos	0.01	-	Fungicide	Organophosphate	Not
Emamectin	0.01	-	Insecticide		Approved
Endosulphan alpha	0.01	-	Insecticide, Acaricide	Organochlorine	Not
Endosulphan beta	0.01	-	Insecticide, Acaricide	Organochlorine	Not
Endosulphan sulphate	0.01	-			
Endrin	0.01	-	Insecticide, Avicide	Organochlorine	Not, banned
Epn	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Epoxiconazole	0.01	-	Fungicide	Triazole	Approved
EPTC	0.01	-	Herbicide	Thiocarbamate	Not
Etaconazole	0.01	-	Fungicide	Conazole	Not
Ethidimuron	0.01	-	Herbicide	Thiadiazolylurea	Not

Ethiofencarb	0.01	-	Insecticide	Carbamate	Not
Ethiofencarb sulfone	0.01	-	Metabolite	Unclassified	Not
Ethiofencarb sulfoxide	0.01	-	Metabolite	Unclassified	Not
Ethion	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Ethiprole	0.01	-	Insecticide	Phenylpyrazole	Not
Ethirimol	0.01	-	Fungicide	Pyrimidinol	Not
Ethofumesate	0.01	-	Herbicide	Benzofuran	Approved
Ethoprophos	0.01	-	Insecticide, Nematicide	Organophosphate	Not
Ethoxyquin	**0.05**	-	Plant growth regulator	Quinoline	Not
Etofenprox	0.01	-	Insecticide	Pyrethroid	Approved
Etoxazole	0.01	-	Insecticide	Diphenyl oxazoline	Approved
Etridiazole	0.01	-	Fungicide	Aromatic hydrocarbon	Approved
Etrimfos	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Famoxadone	**0.05**	-	Fungicide	Oxazole	Approved
Famphur	0.01	-			
Fenamidone	0.01	-	Fungicide	Imidazole	Not
Fenamiphos	0.01	-	Nematicide	Organophosphate	Approved
Fenamiphos sulfone	0.01	-	Metabolite	Unclassified	Not
Fenamiphos sulfoxide	0.01	-	Metabolite	Unclassified	Not
Fenarimol	0.01	-	Fungicide	Pyrimidine	Not
Fenazaquin	0.01	-	Acaricide	Quinazoline	Approved
Fenbuconazole	0.01	-	Fungicide	Triazole	Approved
Fenchlorphos	0.01	-	Insecticide	Organophosphate	Not
Fenchlorphos oxon	0.01	-			
Fenhexamid	0.01	-	Fungicide	Fenhexamid	Approved
Fenitrothion	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Fenoxycarb	0.01	-	Insecticide	Carbamate	Approved
Fenpiclonil	0.01	-	Fungicide	Phenylpyrrole	Not
Fenpropathrin	0.01	-	Insecticide, Acaricide	Pyrethroid	Not
Fenpropidin	0.01	-	Fungicide	Unclassified	Approved

Fenpropimorph	0.01	-	Fungicide	Morpholine	Approved
Fenpyroximate	0.01	-	Insecticide, Acaricide	Pyrazolium	Not
Fenson	0.01	-	Acaricide	Organochlorine	Not
Fensulfothion	0.01	-	Insecticide, Nematicide	Organophosphate	Not
Fenthion	0.01	-	Insecticide	Organophosphate	Not
Fenthion Sulphone? Fenthion sulfone?	0.01	-			
Fenthion Sulphoxide? Fenthion sulfoxide?	0.01	-			
Fenuron	0.01	-	Herbicide	Urea	Not
Fenvalerate	0.01	-	Insecticide, Acaricide	Pyrethroid	Not
Fipronil	0.01	-	Insecticide	Phenylpyrazole	Not
Fipronil sulphone? Fiponil sulfone	0.01	-			
Flamprop isopropyl?	0.01	-			
Flonicamid	0.01	-	Insecticide	Pyridine compound	Approved
Fluazifop-P-Butyl	0.01	-			
Fluazinam	0.01	-	Fungicide	Phenylpyridinamine	Approved
Flucythrinate	0.01	-	Insecticide	Pyrethroid	Not
Fludioxonil	0.01	-	Fungicide	Phenylpyrrole	Approved
Flufenacet	0.01	-	Herbicide	Oxyacetamide	Approved
Flufenoxuron	0.01	-	Insecticide	Benzoylurea	Not
Flumetralin	0.01	-	Plant growth regulator	Unclassified	Approved
Flumioxazin	0.01	-	Herbicide	N-phenylphtalamides	Approved
Flumorph	0.01	-	Fungicide	Morpholine	
Fluometuron	0.01	-	Herbicide	Phenylurea	Approved
Fluopicolide	0.01	-	Fungicide	Benzamide	Approved
Fluopyram	0.01	-	Fungicide	Benzamide, pyramide	Approved
Fluorochloridone	0.01	-	Herbicide	Unclassified	
Fluoxastrobin	0.01	-	Fungicide	Strobilurin	Approved
Fluquinconazole	0.01	-	Fungicide	Triazole	Approved

Fluroxypyr-1-methylheptyl ester	0.01	-	Herbicide	Pyridine compound	
Flurtamone	0.01	-	Herbicide	Pyridazinone	Not
Flusilazole	0.01	-	Fungicide	Triazole	Not
Flutolanil	0.01	-	Fungicide	Oxathiin	Approved
Flutriafol	0.01	-	Fungicide	Triazole	Approved
Fluxapyroxad	0.01	-	Fungicide	Pyrazolium	Approved
Folpet	0.01	-	Fungicide	Phthalimide	Approved
Fonophos	0.01	-	Insecticide	Organophosphate	
Forchlorfenuron	0.01	-	Plant growth regulator	Phenylurea	Approved
Formetanate	0.01	-	Insecticide, Acaricide	Formamidine	Approved
Formothion	**0.05**	-	Insecticide, Acaricide	Organophosphate	Not
Fosthiasate	0.01	-			
Fuberidazole	0.01	-	Fungicide	Benzimidazole	Not
Furalaxyl	0.01	-	Fungicide	Acylalanine	Not
Furathiocarb	0.01	-	Insecticide	Carbamate	Not
Glyphosate	0.01	**22**	Herbicide	Phosphonoglycine	Approved
Haloxyfop etotyl	0.01	-	Herbicide	Aryloxyphenoxypropionate	Not
Haloxyfop Methyl	0.01	-	Herbicide	Aryloxyphenoxypropionate	Approved
Heptachlor	0.01	-	Insecticide	Organochlorine	Not, banned
Heptachlor epoxide	0.01	-	Metabolite	Unclassified	
Heptachlor exo Epoxide	0.01	-			
Heptenophos	0.01	-	Insecticide	Organophosphate	Not
Hexachlorobenzene	0.01	-	Fungicide	Chlorinated hydrocarbon	Not, banned
Hexachlorocyclohexane (alpha)	0.01	-	Insecticide	Organochlorine	Not, banned
Hexachlorocyclohexane (beta)	0.01	-			Not, banned
Hexachlorocyclohexane (delta)	0.01	-			Not, banned
Hexaconazole	0.01	-	Fungicide	Triazole	Not

Hexaflumuron	0.01	-	Insecticide	Benzoylurea	Not
Hexazinone	0.01	-	Herbicide	Triazinone	Not
Hexythiazox	0.01	-	Insecticide, Acaricide	Carboxamide	Approved
Imazalil	0.01	-	Fungicide	Imidazole	Approved
Imibenconazole	0.01	-	Fungicide	Triazole	Not
Imidacloprid	0.01	-	Insecticide	Neonicotinoid	Approved
Indoxacarb	0.01	-	Insecticide	Oxadiazine	Approved
Iodofenphos	0.01	-	Insecticide	Organophosphate	Not
Iprodione	0.01	-	Fungicide, Nematicide	Dicarboximide	Not
Iprovalicarb	0.01	-	Fungicide	Carbamate	Approved
Isazofos	0.01	-	Insecticide	Organophosphate	Not
Isocarbophos	0.01	-	Acarcicide, Insecticide	Organophosphate	Not
Isodrin	0.01	-	Insecticide	Cyclodiene	
Isofenphos	0.01	-	Insecticide	Organophosphate	Not
Isofenphos Methyl	0.01	-	Insecticide	Organophosphate	Not
isomers	0.01	-			
Isomethiozin	0.01	-	Herbicide	Triazinone	
Isoprocarb	0.01	-	Insecticide	Carbamate	Not
Isoprothiolane	0.01	-	Fungicide	Phosphorothiolate	Not
Isoproturon	0.01	-	Herbicide	Urea	Not
Isopyrazam	0.01	-	Fungicide	Pyrazole	Approved
Isothiazolinone	0.01	-			
Isoxaben	0.01	-	Herbicide	Benzamide	Approved
Karbutilate	0.01	-	Herbicide	Carbamate	Not
Kresoxim Methyl	0.01	-	Fungicide	Strobilurin	Approved
Lambda Cyhalothrin	0.01	-	Insecticide	Pyrethroid	Approved
Lenacil	0.01	-	Herbicide	Uracil	Approved
Leptophos	0.01	-	Insecticide	Organophosphate	Not
Lindane	0.01	-	Insecticide, Rodenticide	Organochlorine	Not
Linuron	0.01	-	Herbicide	Urea	Not

Lufenuron	0.01	-	Insecticide	Benzoylurea	Approved
Malaoxon	0.01	-			
Mandipropamid	0.01	-	Fungicide	Mandelamide	Approved
MCPA-thioethyl	0.01	-	Herbicide	Aryloxyalkanoic acid	Approved
Mecarbam	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Mefenacet	0.01	-	Herbicide	Oxyacetamide	Not
Mepanipyrim	0.01	-	Fungicide	Anilinopyrimidine	Approved
Mephosfolan	0.01	-	Insecticide	Organophosphate	Not
Mepronil	0.01	-	Fungicide	Benzanilide	Not
Metaflumizone	0.01	-	Insecticide	Semicarbazone	Approved
Metalaxyl	0.01	-	Fungicide	Phenylamide	Approved
Metamitron	0.01	-	Herbicide	Triazinone	Approved
Metazachlor	0.01	-	Herbicide	Chloroacetamide	Approved
Metconazole	0.01	-	Fungicide, Plant growth regulator	Triazole	Approved
Methabenzthiazuron	0.01	-	Herbicide	Urea	Not
Methacrifos	0.01	-	Insecticide	Organophosphate	Not
Methamidophos	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Methidathion	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Methiocarb	0.01	-	Insecticde, Repellant	Carbamate	Approved
Methiocarb sulfone	0.01	-	NA	Unclassified	
Methiocarb Sulfoxide	0.01	-	NA	Carbamate	Not
Methomyl	0.01	-	Insecticide	Carbamate	Approved
Methoxychlor	0.01	-	Insecticide	Organochlorine	Not
Methoxyfenozide	0.01	-	Insecticide	Diacylhydrazine	Approved
Methyl Paraoxon	0.01	-			
Metobromuron	0.01	-	Herbicide	Urea	Approved
Metolachlor	0.01	-	Herbicide	Chloroacetamide	Not
Metolcarb	0.01	-	Insecticide	Carbamate	Not
Metoxuron	0.01	-	Herbicide	Urea	Not
Metrafenone	0.01	-	Fungicide	Benzophenone	Approved

Metribuzin	0.01	-	Herbicide	Triazinone	Approved
Mevinphos	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Mirex	0.01	-	Insecticide	Organochlorine	Not, banned
Molinate	0.01	-	Herbicide	Thiocarbamate	Not
Monocrotophos	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Monolinuron	0.01	-	Herbicide	Urea	Not
Monuron	0.01	-	Herbicide	Phenylurea	Not
Myclobutanil	0.01	-	Fungicide	Triazole	Approved
Napropamide	0.01	-	Herbicide	Alkanamide	Approved
Neburon	0.01	-	Herbicide	Urea	Not
Nicotine	0.01	-	Insecticide	Plant derived	Not
Nitenpyram	0.01	-	Insecticide	Neonicotinoid	Not
Nitrofen	0.01	-	Herbicide	Diphenyl ether	Not, banned
Nitrothal isopropyl	0.01	-	Fungicide	Unclassified	Not
Novaluron	0.01	-	Insecticide	Benzoylurea	Not
Nuarimol	0.01	-	Fungicide	Pyrimidine	Not
o,p'-DDT	0.01	-	Insecticide	Organochlorine	Not
Octhilinone	0.01	-	Fungicide	Heteroaramatic	Not
Ofurace	0.01	-	Fungicide	Phenylamide	Not
Omethoate	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Orysastrobin	0.01	-	Fungicide	Strobilurin	Not
Oxadiargyl	0.01	-	Herbicide	Oxidiazole	Not
Oxadiazon	0.01	-	Herbicide	Oxidiazole	Not
Oxadixyl	0.01	-	Fungicide	Phenylamide	Not
Oxamyl	0.01	-	Insecticide, Nematicide	Carbamate	Approved
Oxycarboxin	0.01	-	Fungicide	Oxathiin	Not
Oxydemeton-Methyl	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Oxyfluorfen	0.01	-	Herbicide	Diphenyl ether	Approved
p,p-DDD	0.01	-	Insecticide	Organochlorine	
p,p-DDE	0.01	-	NA		NA

p,p-DDT	0.01	-	Insecticide	Organochlorine	Not
Paclobutrazol	0.01	-	Plant growth regulator	Triazole	Approved
Paraoxon	0.01	-	NA		NA
Parathion ethyl	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Parathion methyl	0.01	-	Insecticide, Repellant	Organophosphate	Not
Penconazole	0.01	-	Fungicide	Triazole	Approved
Pencycuron	0.01	-	Fungicide	Phenylurea	Approved
Pendimethalin	0.01	-	Herbicide	Dinitroaniline	Approved
Pentachloroaniline	0.01	-	Metabolite	Unclassified	Not
Pentachlorophenol	0.01	-	Herbicide	Organochlorine	Not
Pentanochlor	0.01	-	Herbicide	Anilide	Not
Permethrin	0.01	-	Insecticide	Pyrethroid	Not
Pethoxamid	0.01	-	Herbicide	Chloroacetamide	Approved
Phenmedipham	0.01	-	Herbicide	Carbamate	Approved
Phenothrin	0.01	-	Insecticide	Pyrethroid	Not
Phenthoate	0.01	-	Insecticide	Organophosphate	Not
Phorate	0.01	-	Insecticide	Organophosphate	Not
Phorate sulfone	0.01	-	NA		NA
Phorate sulfoxide	0.01	-	Metabolite	Organophosphate	Not
Phosalone	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Phosfolan	0.01	-	Insecticide	Organophosphate	Not
Phosmet	0.01	-	Insecticide	Organophosphate	Approved
Phosphamidon	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Phoxim	0.01	-	Insecticide	Organophosphate	Not
Phthalimide	0.01	-	NA		NA
Picoxystrobin	0.01	-	Fungicide	Strobilurin type-methoxyacrylate	Not
Piperonyl Butoxide	0.01	**22**	Not a plant protection product	Cyclic aromatic	NA
Pirimicarb	0.01	-	Insecticide	Carbamate	Approved
Pirimicarb desmethyl	0.01	-	NA		NA

Pirimiphos Ethyl	0.01	-	Insecticide	Organophosphate	Not
Pirimiphos methyl	0.01	**10**	Insecticide	Organophosphate	Approved
Pretilachlor	0.01	-	Herbicide	Chloroacetamide	Not
Prochloraz	0.01	-	Fungicide	Imidazole	Approved
Procymidone	0.01	-	Fungicide	Dicarboximide	Not
Profenofos	0.01	-	Insecticide	Organophosphate	Not
Promecarb	0.01	-	Insecticide	Carbamate	Approved
Prometon	0.01	-	Herbicide	Methoxytriazine	Not
Prometryn	0.01	-	Herbicide	Triazine	Not
Propachlor	0.01	-	Herbicide	Chloroacetamide	Not
Propamocarb	0.01	-	Fungicide	Carbamate	Approved
Propanil	0.01	-	Herbicide	Anilide	Not
Propaphos	0.01	-	Insecticide	Organophosphate	Not
Propaquizafop	0.01	-	Herbicide	Aryloxyphenoxypropionate	Approved
Propargite	0.01	-	Acaricide	Sulphite ester	Not
Propazine	0.01	-	Herbicide	Triazine	Not
Propetamphos	0.01	-	Insecticide	Organophosphate	Not
Propham	0.01	-	Herbicide, Plant growth regulator	Carbamate	Not
Propiconazole	0.01	-	Fungicide	Triazole	Not
Propoxur	0.01	-	Insecticide	Carbamate	Not
Propyzamide	0.01	-	Herbicide	Benzamide	Approved
Proquinazid	0.01	-	Fungicide	Quinazolinone	Approved
Prosulfocarb	0.01	-	Herbicide	Thiocarbamate	Approved
Prothioconazole desthio	0.01	-	Fungicide	Triazolinthione	Not
Prothiofos	0.01	-	Insecticide	Organophosphate	Not
Pymetrozine	0.01	-	Insecticide	Pyridine	Not
Pyraclostrobin	0.01	-	Fungicide, Plant growth regulator	Strobilurin	Approved
Pyraflufen ethyl	0.01	-	Herbicide	Phenylpyrazole	Approved
Pyrazophos	0.01	-	Fungicide	Phosphorothiolate	Not
Pyrethrin	0.01	-	Insecticide	Plant derived	Approved

Pyridaben	0.01	-	Insecticide, Acaricide	Pyridazinone	Approved
Pyridaphenthion	0.01	-	Insecticide, Acaricide		Not
Pyrifenox	0.01	-	Fungicide	Pyridine	Not
Pyrimethanil	0.01	-	Fungicide	Anilinopyrimidine	Approved
Pyrimidifen	0.01	-			Not
Pyriproxyfen	0.01	-	Insecticide	Unclassified	Approved
Quinalphos	0.01	-	Insecticide	Organophosphate	Not
Quinoxyfen	0.01	-	Fungicide	Quinoline	Not
Quintozene	0.01	-	Fungicide	Chlorophenyl	Not
Quizalofop-ethyl	0.01	-	Herbicide	Aryloxyphenoxypropionate	Not
Resmethrin	0.01	-	Insecticide	Pyrethroid	Not
Rotenone	0.01	-	Insecticide		Not
S421	0.01	-			
Secbumeton	**0.05**	-	Herbicide	Methoxytriazine	Not
Silafluofen	0.01	-	Insecticide	Pyrethroid	Not
Simazine	0.01	-	Herbicide	Triazine	Not
Simeconazole	0.01	-	Fungicide	Conazole	Not
Spinetoram	0.01	-	Insecticide	Spinosym	Approved
Spirodiclofen	0.01	-	Insecticide, Acaricide	Tetronic acid	Approved
Spiromesifen	0.01	-	Insecticide, Acaricide	Tetronic acid	Approved
Spirotetramat	0.01	-	Insecticide	Tetramic acid	Approved
Spiroxamine	0.01	-	Fungicide	Morpholine	Approved
Sulfallate	0.01	-	Herbicide	Thiocarbamate	Not
Sulfentrazone	0.01	-	Herbicide	Aryl triazolinone	Not
Sulprofos	0.01	-	Insecticide	Organophosphate	Not
Tau-Fluvalinate	0.01	-	Insecticide	Synthetic pyrethroid	Approved
Tebuconazole	0.01	-	Fungicide	Triazole	Approved
Tebufenozide	0.01	-	Insecticide	Diacylhydrazine	Approved
Tebufenpyrad	0.01	-	Acaricide	Pyrazolium	Approved
Tebupirimiphos	0.01	-			

Tecnazene	0.01	-	Fungicide, Plant growth regulator	Chlorophenyl	Not
Teflubenzuron	0.01	-	Insecticide	Benzoylurea	Approved
Tefluthrin	0.01	-	Insecticide	Pyrethroid	Approved
Temephos	0.01	-	Insecticide	Organophosphate	Not
Terbacil	0.01	-	Herbicide	Uracil	Not
Terbufos	0.01	-	Insecticide	Organophosphate	Not
Terbufos sulfone	0.01	-			
Terbufos sulfoxide	0.01	-			
Terbumeton	0.01	-	Herbicide	Triazine	Not
Terbuthylazine	0.01	-	Herbicide	Triazine	Approved
Terbutryn	0.01	-	Herbicide	Triazine	Not
Tetrachlorvinphos	0.01	-	Insecticide	Organophosphate	Not
Tetraconazole	0.01	-	Fungicide	Triazole	Approved
Tetradifon	0.01	-	Insectcide, Acaricide	Bridged diphenyl	Not
Tetraethyl dithiopyrophosphate	0.01	-	Insecticide, Acaricide	Organophosphate	Not
Tetramethrin	0.01	-	Insecticide	Pyrethroid	Not
Tetrasul	0.01	-	Acaricide	Bridged diphenyl	Not
Thiabendazole	0.01	-	Fungicide	Benzimidazole	Approved
Thiacloprid	0.01	-	Insecticide	Neonicotinoid	Approved
Thiamethoxam	0.01	-	Insecticide	Neonicotinoid	Approved
Thiazafluron	0.01	-	Herbicide	Thiadiazolylurea	Not
Thidiazuron	0.01	-	Plant growth regulator	Phenylurea	Not
Thiobencarb	0.01	-	Herbicide	Thiocarbamate	Not
Thiocyclam	0.01	-	Insectcide	Unclassified	Not
Thiodicarb	0.01	-	Insectcide	Carbamate	Not
Thiofanox	0.01	-	Insectcide	Carbamate	Not
Thiometon	0.01	-	Insectcide, Acaricide	Organophosphate	Not
Thiophanate Methyl	0.01	-	Fungicide	Benzimidazole	Approved
Tolclofos methyl	0.01	-	Fungicide	Chlorophenyl	Approved

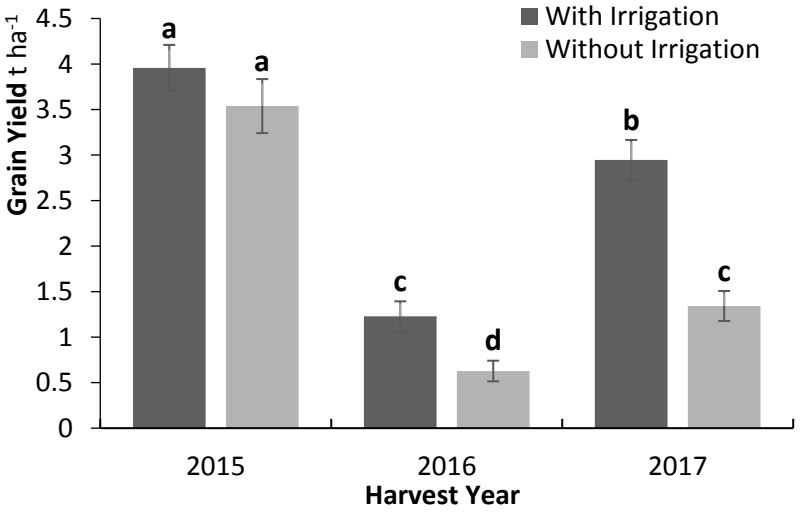
Tolylfluanid	0.01	-	Fungicide, Acaricide	Sulphamide	Not
Triadimefon	0.01	-	Fungicide	Triazole	Not
Triadimenol	0.01	-	Fungicide	Triazole	Approved
Triallate	0.01	-	Herbicide	Thiocarbamate	
Triazamate	0.01	-	Insectcide	Carbamoyltriazole	Not
Triazophos	0.01	-	Insectcide, Acaricide	Organophosphate	Not
Tribenuron methyl	0.01	-	Herbicide	Sulfonylurea	Not
Trichlorfon	0.01	-	Insectcide	Organophosphate	Not
Tridemorph	0.01	-	Fungicide	Morpholine	Not
Trietazine	0.01	-	Herbicide	Triazine	Not
Trifloxystrobin	0.01	-	Fungicide	Strobilurin	Approved
Trifloxysulfuron	0.01	-	Herbicide	Sulfonylurea	Not
Triflumizole	0.01	-	Fungicide	Imidazole	Approved
Triflumuron	0.01	-	Insectcide	Benzoylurea	Approved
Trifluralin	0.01	-	Herbicide	Dinitroaniline	Not
Triflusulfuron-methyl	0.01	-	Herbicide	Sulfonylurea	Approved
Triforine	0.01	-	Fungicide, Acaricide	Piperazine	Not
Triticonazole	0.01	-	Fungicide	Triazole	Approved
Uniconazole	0.01	-	Plant growth regulator	Triazole	Not
Vamidothion	0.01	-	Insectcide, Acaricide	Organophosphate	Not
Vernolate	0.01	-	Herbicide	Thiocarbamate	Not
Vinclozolin	0.01	-	Fungicide	Oxazole	Not
Zoxamide	0.01	-	Fungicide	Benzamide	Approved

*LOQ is the lowest concentration at which the analyse can not only be reliably detected but at which some predefined goals for bias and imprecision are met

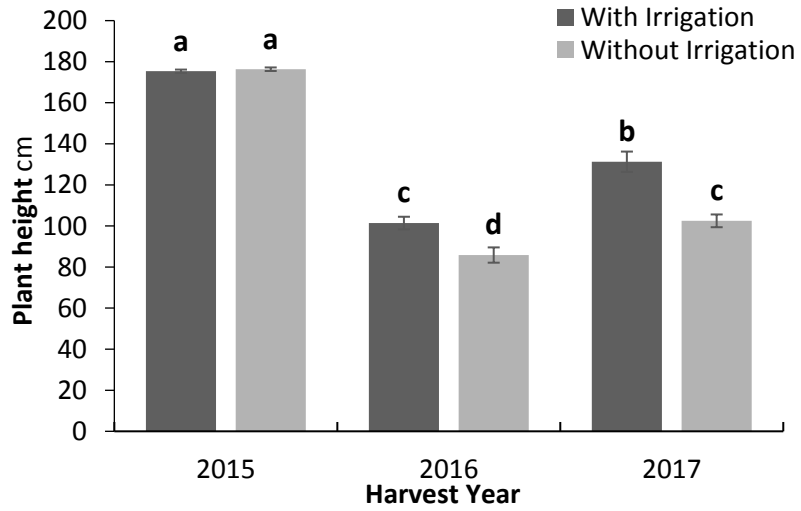
Appendix 5.1 Effects of Harvest year, irrigation and fertiliser type on spelt wheat grain yield, harvest index plant height, stem lodging, yield components, crude protein content SPAD as an estimation of leaf chlorophyll content

	Grain Yield	**Harvest Index**	**Plant Height GS62***	**Lodging**	**Tillers/m²**	**Ears/m²**	**Grain/hull**	**TGW**	**SPAD GS39**	**SPAD GS50**	**SPAD GS62**
Means ±SE	t ha^{-1}	%	cm	%			%	g			
Year (n=24)											
2015	3.7±0.2 **a**	19.2±0.5 **a**	176±1 **a**	15 ±4	434 ±10 **a**	330 ±10 **a**	70.0 ±0.4 **a**	43.4 ±0.7 **a**	42.0 ±0.3	41.9 ±0.2 **a**	39.4 ±0.3
2016	0.9±0.1 **c**	10.9±0.9 **b**	94±3 **c**	13 ±3	307 ±20 **b**	173 ±13 **c**	58.4 ±2.1 **b**	35.8 ±0.6 **c**	-	38.5 ±1.0 **b**	-
2017	2.1±0.2 **b**	21.8±1.4 **a**	117±4 **b**	7 ±2	332 ±12 **b**	264 ±14 **b**	67.1 ±0.6 **a**	38.7 ±0.9 **b**	39.8 ±1.0	42.8 ±0.6 **a**	43.6 ±0.8
Irrigation (n=36)											
With	2.7±0.2	18.3±1.0	136±5	19 ±2	383 ±13	277 ±14	67.2 ±0.9	40.6± 0.7	40.0 ±0.7	40.2 ±0.7	41.0 ±0.6
Without	1.8±0.2	16.3±1.2	122±7	5 ±1	333 ±16	240 ±16	63.2 ±1.6	38.0 ±0.9	41.7 ±0.8	41.9 ±0.5	42.0 ±0.9
Fertiliser type (n=24)											
CHI*	2.2±0.3	17.8±1.4 **ab**	127±8	10 ±2	355 ±21	267 ±20	64.6 ±2.0 **ab**	38.7 ±1.0 **b**	39.8 ±0.9	40.6 ±0.5 **b**	40.8 ±0.7
MIN*	2.3±0.4	15.3±1.4 **b**	132±8	14 ±3	363 ±20	252 ±23	63.5 ±1.8 **b**	38.3 ±1.0 **b**	43.4 ±0.7	43.1 ±0.7 **a**	42.9 ±1.2
SHE*	2.3±0.3	18.8±1.3 **a**	127±7	11 ±3	355 ±15	257 ±12	67.5 ±0.8 **a**	40.9 ±0.8 **a**	39.4± 0.7	39.4 ±0.9 **b**	40.8 ±0.7
ANOVA											
Main effects											
Year (YR)	**0.0001**	**0.0007**	**<.0001**	0.0653	**0.0007**	**0.0002**	**0.0009**	**0.0007**	NS	**0.0387**	**0.0153**
Irrigation (IR)	**0.0006**	0.0748	**0.0002**	**0.0003**	**0.0048**	**0.0282**	**0.0131**	**0.0113**	**0.0333**	**0.0087**	NS
Fertiliser type (FT)	NS	**0.0247**	0.0797	NS	NS	NS	**0.0441**	**0.0279**	**<.0001**	**<.0001**	**0.019**
Interactions											
YR x IR	**0.0401** [1]	*0.0531*	**0.0019** [2]	**0.0163** [5]	**0.0205** [6]	NS	NS	NS	NS	**0.0054**	**0.0354**
YR x FT	NS	*0.0848*	**0.0028** [3]	NS	**0.0013** [7]	**0.0068** [8]	*0.0664*	NS	**0.0006**	**0.0388**	**0.0007**
IR x FT	NS	NS	**0.0475** [4]	NS	NS	NS	NS	NS	NS	NS	NS
YR x IR x FT	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

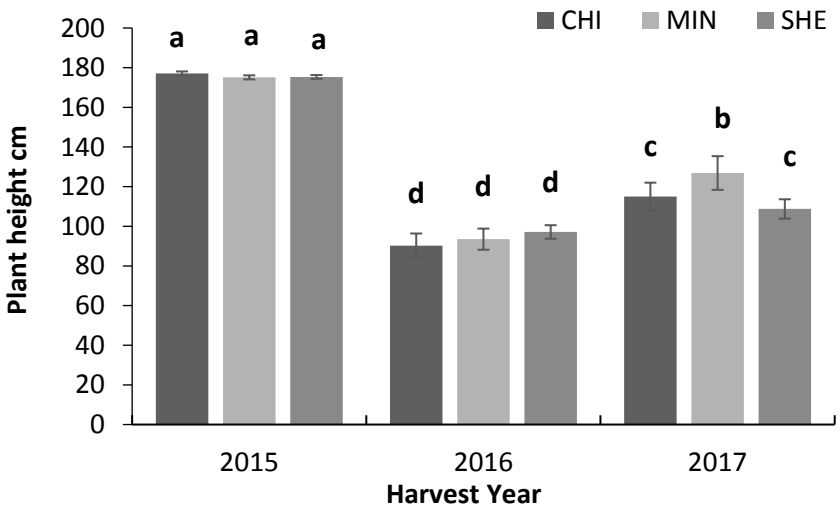
*GS 62: growth stage 62 start of flowering; *CHI: chicken manure; *MIN: mineral fertiliser; *SHE: sheep manure; Means that are followed by the same letter within each column are not significant different (general linear hypothesis test p<0.05);
1See figures 5.1.1 for interaction means ±SE; 2See figure 5.1.2 for interaction means ±SE; 3See figure 5.1.3 for interaction means ±SE; 4See figure 5.1.4 for interaction means ±SE; 5See figure 5.1.5 for interaction means ±SE;



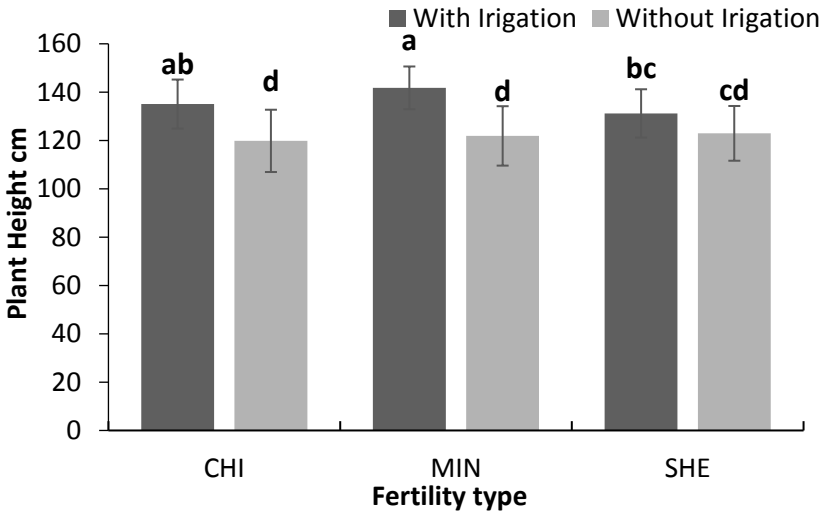
Appendix 5.2 Effects of interaction between harvest year and irrigation on spelt wheat grain yield.



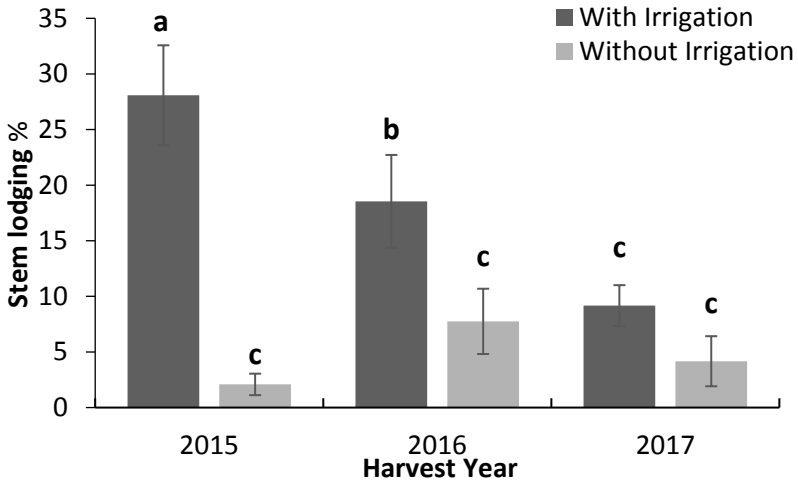
Appendix 5.3 Effects of interaction between harvest year and irrigation on spelt wheat plant height at GS62.



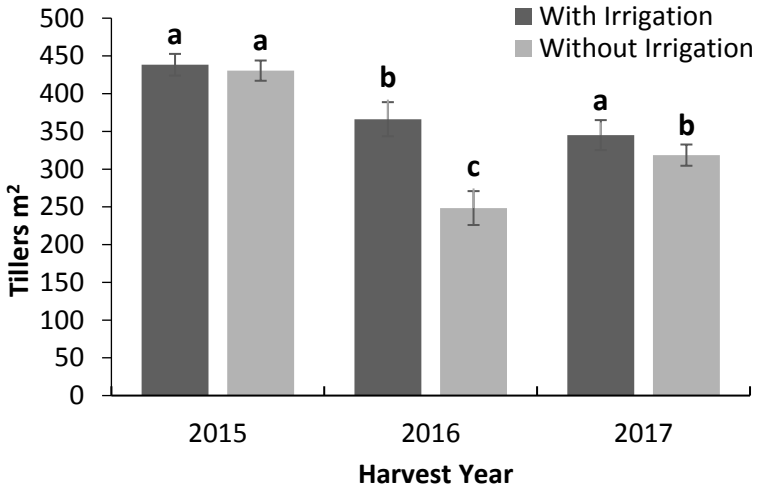
Appendix 5.4 Effects of interaction between harvest year and fertiliser type on spelt wheat plant height at GS62.



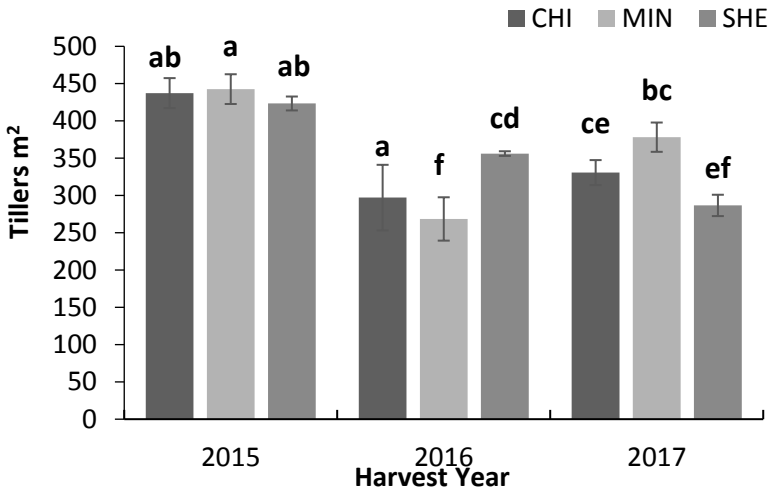
Appendix 5.5 Effects of interaction between irrigation and fertiliser type on spelt wheat plant height at GS62.



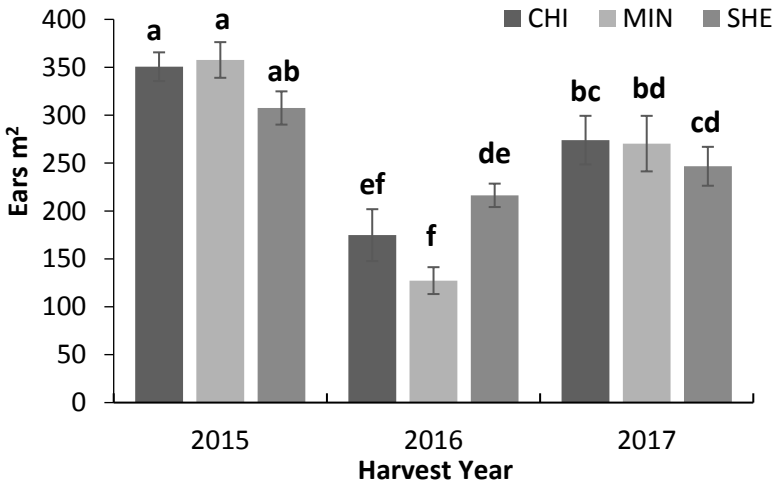
Appendix 5.6 Effects of interaction between harvest year and irrigation on spelt wheat straw lodging at GS85.



Appendix 5.7 Effects of interaction between harvest year and irrigation on number of tillers of spelt wheat at GS85.



Appendix 5.8 Effects of interaction between harvest year and variety choice and fertiliser type on number of tillers of spelt wheat at GS85.



Appendix 5.9 Effects of interaction between harvest year and variety choice and fertiliser type on number of ears of spelt wheat at GS85.

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