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Cereal whole grain for human nutrition.

Influence of genetic, environmental and technological factors on content and composition of specific bioactive compounds

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I dedicate this thesis

to my family and my grandparents,

for their constant support and unconditional love.

I love you all dearly.

Roberto Ciccoritti

Content

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List of publications

This thesis is based on the research work described in the following papers:

Ciccoritti R., Carbone K., Pasquini M., Pogna N., Sgrulletta D., Nocente F.(2013). *"Recenti sviluppi sulle proprietà di estratti di 5-n-alchilresorcinoli da cariossidi di frumento duro*" Atti 9°Convegno AISTEC, Un mondo di cereali: Potenzialità e sfide. 12-14 giugno Bergamo p.56-61 ISBN 978-88-90669680-1-

Del Frate V., Terracciano G., Cammerata A., Nocente F. e **Ciccoritti R.** (2013) *"Variazioni di composti bioattivi presenti nella granella di frumento duro in seguito al trattamento di parbolizzazione*". Riassunto 11° Congresso Italiano di Scienze e Tecnologia degli Alimentari. 22-23 maggio Milano p.22

Bellato S., **Ciccoritti R.,** Del Frate V., Sgrulletta D., Carbone K. (2013). "*Influence of genotype and environment on the content of 5-n alkylresorcinol on the antiradical activity of whole grain durum wheat grain*". Journal of Cereal Science vol 57: 162-169. ISSN 0733-5210

Ciccoritti R., Bellato S., Carbone K., Sgrulletta D. (2013). "*Content and relative composition of some phytochemicals in diploid, tetraploid and hexaploid Triticum species with potential nutraceutical propertis*". Journal of Cereal Science vol 57: 200-206. ISSN 0733-5210

Nocente F., **Ciccoritti R.**, Sereni L., Matere A., Sgrulletta D., Pasquini M. (2012) *"Protective effect of bioactive compounds extracted from wheat whole grain against different FHB causal agent*" Abstract of International MPU Workshop Plant protection for the quality and safety of the mediterranean diet. p.36

Ciccoritti R., Scalfati G., Cammerata A. and Sgrulletta D. (2011). "*Variation in Content and Extractability of Durum Wheat (Triticum turgidum L. var durum) Arabinoxilans Associated with Genetic and Environmental Factors*". International Journal of Molecular Sciences vol 12: 4536-4549. ISSN 1422-0067

Bellato S., **Ciccoritti R.,** Scalfati G., Del Frate V., Cammerata A., Gazza L., Sgrulletta D (2010). "*Effetto del trattamento di parboilizzazione sui composti bioattivi della granella dei cereali"* Atti del 8° congresso nazionale di Chimica degli Alimenti, Settembre: 183-186 ISBN 978-88-86208-65-9

Roberto Ciccoritti

Abbreviations

AA	Antiradical Activity
AACC	American Association of Cereal Chemistry
ANOVA	Analysis of Variance
AX	Arabinoxylans
AOAC	Association of Official Analytical Chemists
AR	5-n-alkylresorcinol
ARIC	American Reliable Insurance Company
CAC	Codex Alimentarius
CoA	Coenzyme A
CWM	Cell Wall Material
CTE	Catechin Equivalents
D.F.	Degree of Freedom
d.m.	Dry Matter
DPPH	1,1-Diphenyl-2-Picrylhydrazyl Radical
EC50	Half maximal effective concentration
EI	Electronic Ionizations
eV	electron Volt
F-C	Folin–Ciocalteu
FOS	FructOligoSaccharides
GC-FID	Gas Chromatography Flame Ionization Detector
GC-MS	Gas Chromatography Mass Spectrometry
Hz	Hertz
IDF	Insoluble Dietary Fiber
IS	Internal Standard
MW	Molecular Weight

Roberto Ciccoritti

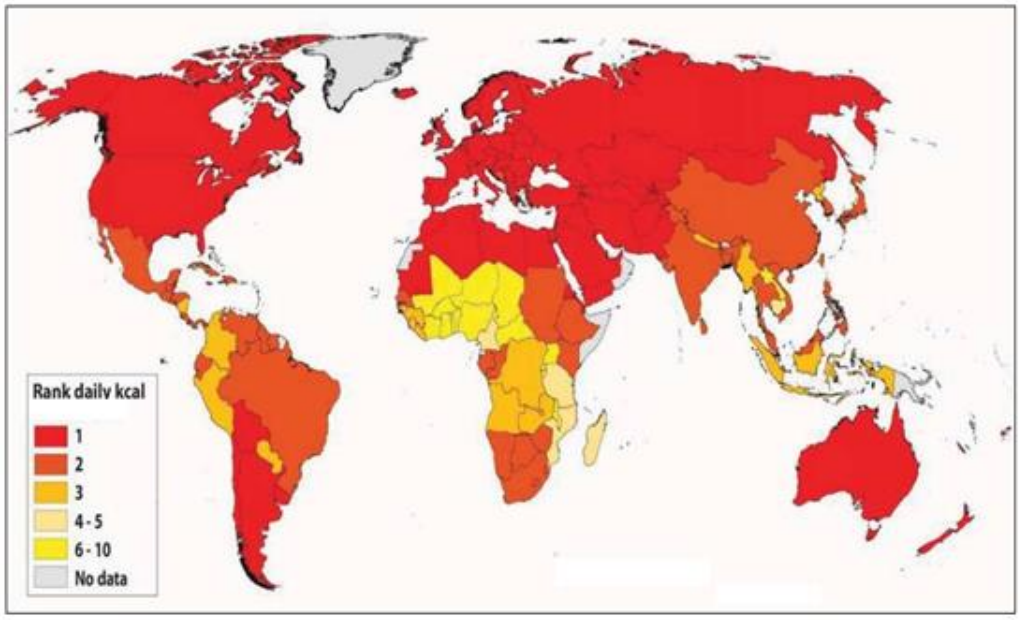
m/z	mass-to-charge ratio
HI	Hydrothermal Processed
PCA	Principal Component Analysis
PC1	First Principal Component
PC2	Second Principal Component
SCFAs	Short Chain Fatty Acids
SDF	Soluble Dietary Fiber
SKCS	Single Kernel Classification System
SSP	Sub Specie
s.l.	sea level
STP	Soluble Total Phenols
TOAX	Total Arabinoxylans
TDF	Total Dietary Fiber
TFA	Tri Fluoro Acetic
TIC	Total Ion Current
TMCS	Trimethylchlorosilane
WEAX	Water Extractable Arabinoxylans
WUAX	Water Unextractable Arabinoxylans

Roberto Ciccoritti

1 Introduction

Cereal products are one of the most important staple foods for humans and have been so for thousands of years. Cereal-based foods are consumed worldwide (Figure 1) with more than 2.3 billion tons of cereals produced annually (FAOSTAT, 2013). The cereal grain cultivation is associated with the development of civilization and now represents the base of world trade. Even if different grains are consumed in the different parts of the world, cereal foods, in general, are an essential component of the diet across cultures (Panatta, 1997).

Figure 1 Cereal crop world consumption (from CYMIT web site).

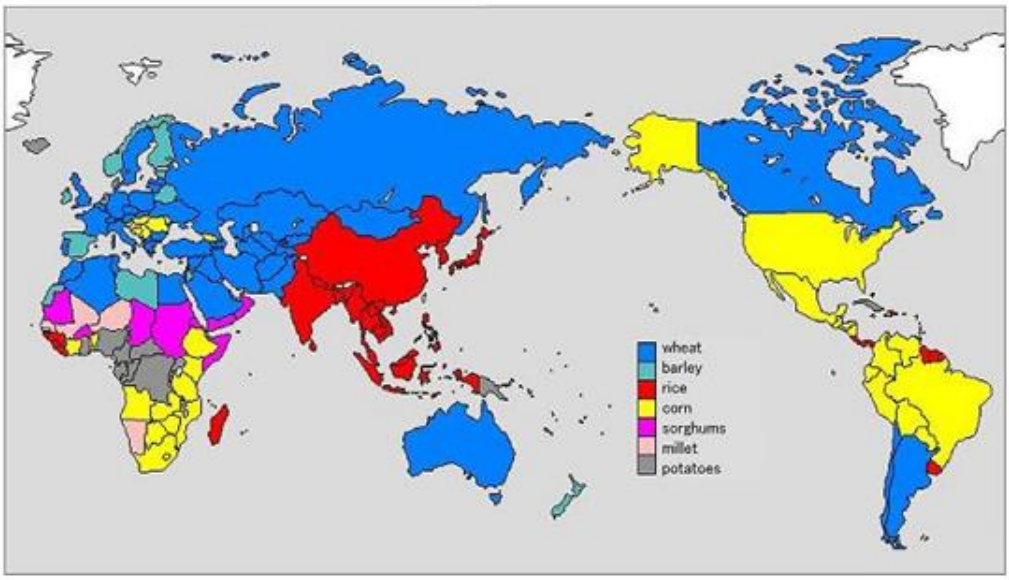


The major crops cultivated in the world are wheat corn and rice (Figure 2), while in temperate climates, such as Europe, wheat, rice, corn, barley, oats, and rye are the principal cereal cultivated crops.

In Europe, the average annual consumption of cereal grains is 131 kg *pro capite*, wheat making up the majority of it (108 kg/capita/year), whereas in Asia, about half of the annual cereal consumption is rice. Wheat and rice are the most important cereals with respect to human nutrition, whereas corn is important in particular in Central and South America, and sorghum and millets in Africa (Kuijsten *et al.*, 2005).

Roberto Ciccoritti

Figure 2 Main crops in every country in the world (from FAOSTAT 2004).



Cereals and cereal products are an important source of carbohydrates, proteins and fiber, as well as of significant amounts of micronutrients such as vitamins (E and B), sodium, magnesium and zinc. Cereal whole grains also contain significant amounts of "bioactive" compounds which may provide health benefits to the consumers; as a consequence, in these last years it is growing the interest on the phytochemical bioavailability (McKevitt, 2004) and on the synergistic effects between whole grains and fruits and vegetables (Liu, 2007) associated with their health benefits.

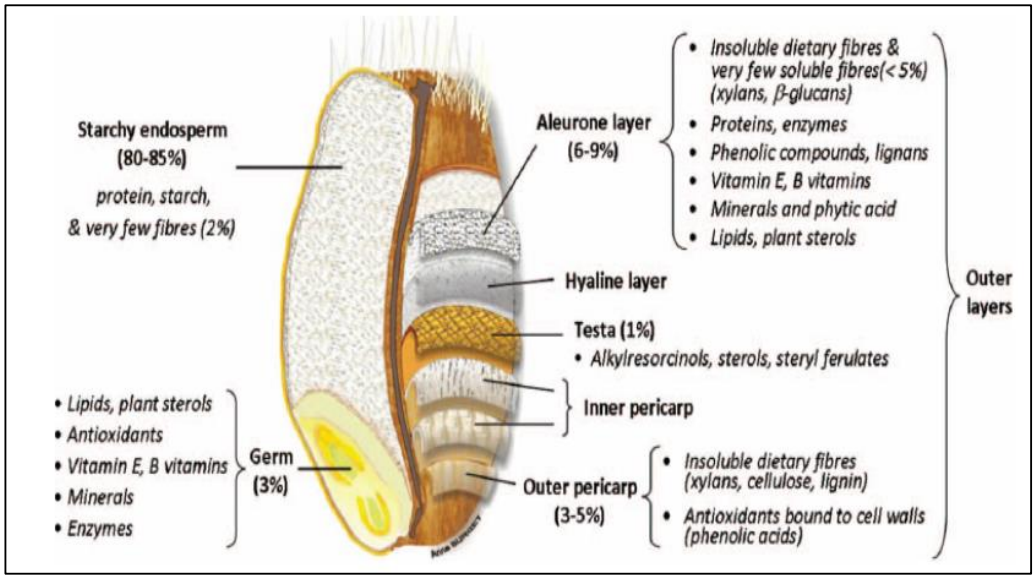
The concept that diets low in meat and high in cereals and legumes are beneficial for health is now widely accepted. As matter of fact, epidemiological studies have shown the protective effect of whole grain consumption for the prevention of various chronic diseases (cardiovascular disease, type 2 diabetes) as well as of some gastrointestinal cancers and obesity (Flight and Clifton, 2006).

1.1 Cereal Whole Grain

Grains, commonly referred to as 'cereals', are the edible seeds of plants belonging to the cereal grass family *Gramineae* (Kellogg, 1998). The grass family includes about 10,000 species, and it encompasses tremendous morphological, physiological, ecological, and genetic diversity. The *Gramineae* family includes all the major cereals, such as wheat, corn and rice, and most of the minor cereals as

well, such as rye, barley, oat, common millet, finger millet, teff spelt, emmer, einkorn and kamut this last three are often referred as 'ancient' grains (Mercader *et al*., 2009). Generally the cereal kernel can be divided in three parts: bran (outer layers), endosperm and germ (Figure 3).

Figure 3. General scheme of wheat kernel (adapted from "Surget and Barron 2005").



In wheat the endosperm accounts for the majority of the wheat kernel or caryopsis (80-85%). The cells in the endosperm are specialized in the storage of starch (80%) and proteins (about 13%) that will function as source of energy for the embryo during germination (Lindsay, 2005).

The germ represents the smallest portion (2-3%) of the wheat grain and consists of the embryonic axis and scutellum. It contains lipids, small amounts of protein, minerals and bioactive compounds of lipophilic nature such as vitamin E, phytosterols and some phenols. The pericarp, the outermost fraction of the wheat kernel comprising about 10-15% of the kernel weight, has the main physiological function of protecting the seed and consists of multiple layers (FAO 2009). From the inner layer to the exterior of the wheat kernel there are: the aleurone layer, the hyaline layer (nucellar epidermis), the testa or seed coat, the inner pericarp (cross and tube cells), and the outer pericarp. The chemical composition of cereals may vary depending on the variety and environmental growing conditions (Hammery *et al*.,

Roberto Ciccoritti

2009). Generally the fiber and protein content (except oat) of wheat is higher, while lower fat amount was reported, on average, than that of other cereals (Table 1)

Table 1. Average composition of cereal grains (%) (from: "NIIR Board 2006").

	Rice	Wheat	Maize	Sorghum	Barley	Oats	Rye
Moisture	12.0	12.5	13.8	11.0	11.1	8.3	11.0
Protein	7.5	12.3	8.9	11.0	8.2	14.2	12.1
Fat	1.9	1.8	3.9	3.3	1.0	7.4	1.7
Fiber	0.9	2.3	2.0	1.7	0.5	1.2	2.0
Ash	1.2	1.7	1.2	1.7	0.9	1.9	1.8

1.1.1 Whole Grain Definition

Grain-based foods are generally grouped as whole grain or refined grain. In 1999 the Board of Directors of AACC approved and accepted the following definition of whole grains:

"Whole-grains shall consist of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components the starchy endosperm, germ, and bran are present in the same relative proportions as they exist in the intact caryopsis".

Whole grains are low in fat, and are important sources of protein, dietary fiber, vitamins (especially B and E group), minerals (magnesium, zinc) and many bioactive phytochemicals, such as arabinoxylans, alkylresorcinols, lignans, flavonoids, ferulic acid, and anthocyanins (Schlemmer *et al*., 2009; Poutanen *et al*., 2012).

Wholemeal is produced by milling whole grains to a finer particle size. Wholemeal is defined as:

"*containing all the milled constituents of the grain in such proportions that it represents the typical ratio of those fractions occurring in the whole cereal*."

More recent evidences also indicate that cereals contain significant quantities of phytochemicals, such as antioxidants and phytoestrogens, which may significantly contribute to reported health benefits of whole grain consumption. In most cases, these beneficial compounds are concentrated in outer layers (bran) of the grain. In

world-wide and Europe most cereal products are obtained by milling kernels after removing bran and germ, the two parts containing the most bioactive components (vitamins, minerals, antioxidants, phytoestrogens and fiber, soluble and insoluble). (Slavin *et al.*, 2001; Jones, 2007). In the last ten years, consumers are (re)discovering whole grain based products, and food industries have substantially increased their efforts in producing cereal based foods reducing the removal of the bran in order to minimize losses of healthy constituents present in the intact grains. Table 2 evidences the markedly different composition between refined grain and wheat whole grain.

Table.2 Compositional differences between whole and refined wheat (from Slavin *et al*., 1999).

Component	Wholewheat	Refined wheat
Bran (%)	14	<0.1
Germ (%)	2.5	<0.1
Total dietary fiber (%)	13	3
Insoluble dietary fiber (%)	11.5	1.9
Soluble dietary fiber (%)	1.1	1.0
Protein (%)	14	14
Fat (%)	2.7	1.4
Starch and sugar (%)	70	83
Total minerals (%)	1.8	0.6
Selected minerals		
Zinc (µg/g)	29	8
Iron (µg/g)	35	13
Selenium (µg/g)	0.06	0.02
Selected vitamins		
Vitamin B-6 (mg/g)	7.5	1.4
Folic acid (mg/g)	0.57	0.11
Phenolic compounds		
Ferulic acid (mg^{-2}/g)	5	0.4
β-tocotrientol (µg/g)	32.8	5.7
Phytate phosphorus (mg/g)	2.9	0.1

The nutrient content of refined flour is determined by the 'extraction rate' (rate of semolina flour in respect of initial grain).

Whole grain foods may be nutritionally preferable overall because furnish valuable nutrients and fiber to a balanced diet, however refined grain-based foods generally have a lower phytate content, which can improve mineral bioavailability. As it is

Roberto Ciccoritti

well known, in fact, dietary fiber and the associated substances have shown *in vitro* mineral binding; many reports indicated that the inhibition of mineral absorption could depend on the type of dietary fiber used (Bosscher *et al.* 2003; Frontella *et al* 2011); in addition data confirmed that the mineral availability could be favored with a more balanced diet wide variety of foods from all the food groups.

1.1.2 Role of Whole Grain in Human Nutrition

Since most of the health-promoting components of grain (fiber, minerals, vitamins, phytoestrogens, etc.) are situated in bran and germ, whole grain consumption can determine higher health benefits compared to refined grain products that contain only the inner parts of the grain. Recent research has shown that the benefits of whole grain are not simply associated with the high fiber content, but can be also related to the presence of other biologically active compounds and to synergistic effects between dietary fiber and various micronutrients. Epidemiological studies in the US and Europe consistently report that consumption of whole grain foods reduces overall disease risk (Jacobs *et al*., 2007; Steffen *et al*., 2005). For example, the ARIC study in the US, which followed more than 15,000 individuals for 11 years, found that those consuming the highest amount of whole grain (three serves a day) had a 23% reduction in mortality compared to those consuming an average of 0.1 serves a day (Williams, 2010). According to the University of Wollongong's National Centre for Excellence in Functional Foods, eating 1-2 serves of whole grain foods /day provides comparable disease risk reduction (in the order of 20-30% of total mortality, cardiovascular disease, diabetes, stroke and some cancers) to that observed for 5-6 serves of fruit and vegetables.

Potential mechanisms proposed for this protective effect are:

1) soluble dietary fiber (SDF) in the intestine reduce dietary fat and cholesterol uptake, alter cholesterol metabolism (circulating cholesterol concentrations) reducing cardiovascular disease risk (Slavin, 2003). Additionally, SDF mediate insulin and glucose responses. Although lower glycaemic load and glycaemic index have been linked to diabetes and obesity, risk of cancers such as colon and breast cancer have also been linked to high intake of readily-available carbohydrate (De Mura, 2009);

2) phytochemicals that act as antioxidants, including trace minerals and phenolic compounds, have potential hormonal effects and for this reason have been linked to

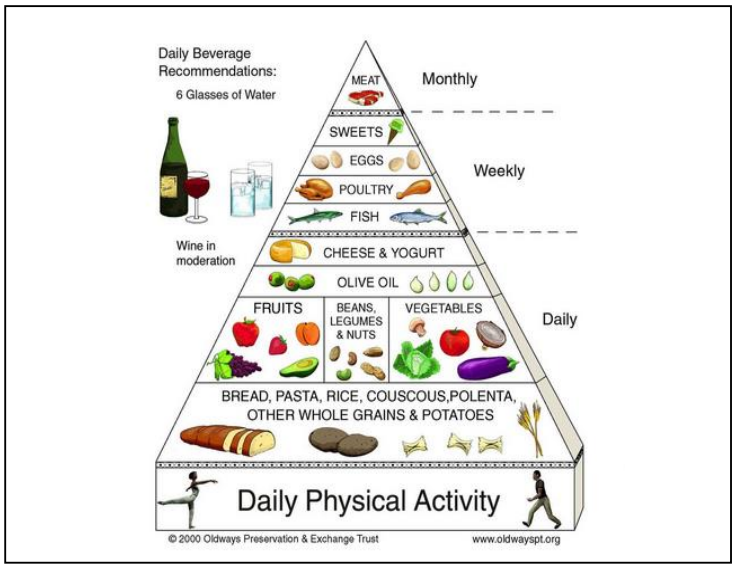
disease prevention against different forms of colon cancer. They may also protect against diseases for their binding of carcinogen substances and reduction of the oxidative stress;

3) whole grains contain many other nutritional compounds: phytate, phyto-estrogens such as lignan, plant stanols and sterols, and vitamins and minerals. Some of them, such as dietary fiber, resistant starch, and oligosaccharides, affect the gut environment. Clearly, the several protective substances present in whole grain allow to justify the advice to consume additional whole grain foods (Wattenberg, 1985).

1.1.3 Whole Grain Dietary Recommendations

The Guidelines for a healthy diet (INRAN, Rev.2003) confirm the importance of cereals, legumes, vegetables and fruit as essential components of a healthy diet. In particular, as highlighted by the "Food Pyramid", which places them at its base (Figure 4), the cereal-based foods are important because they provide complex carbohydrates (mainly starch and fiber), but also vitamins, minerals and other substances of great interest to health.

Figure 4 Food pyramid. (from: www.oldwayspt.org).



In addition, cereals and especially legumes are also good sources of good biological value proteins. Consumption of whole grain is an integral part of the

Roberto Ciccoritti

recommended daily diet in many Western countries and the need to promote the consumption of whole grain has been recognized as one of the targets of nutrition education and of health promotion campaigns (Kushi *et al*., 1999).

Before 2005 US Dietary Guidelines included the recommendation to consume three or more once equivalents of whole grain products each day (about half of the recommended grain serves). Before 2007 revision of Canada's Food Guide it also recommended a daily consume of at least half of the grain products as whole grain.

There has been a recent recommendation for four servings of whole grain each day in Denmark. Since the specific US Public Health Nutrition Guidelines for whole grain were introduced in 2005 there has been a 20% increase in whole grain consumption amongst Americans from 2005-2008. There is, however, no official Italian recommendation that quantifies the amount of whole grain foods to include in a healthy diet each day. As matter of fact INRAN Nutritional Guide recommends to eat whole grain whenever it is possible. Finally, "Wholegrains Council" states "that a portion of food-based grains is such if it contains at least 16 grams of whole grain or whole grain flours" (Williams, 2010).

1.2 Bioactive compounds of cereal whole grain

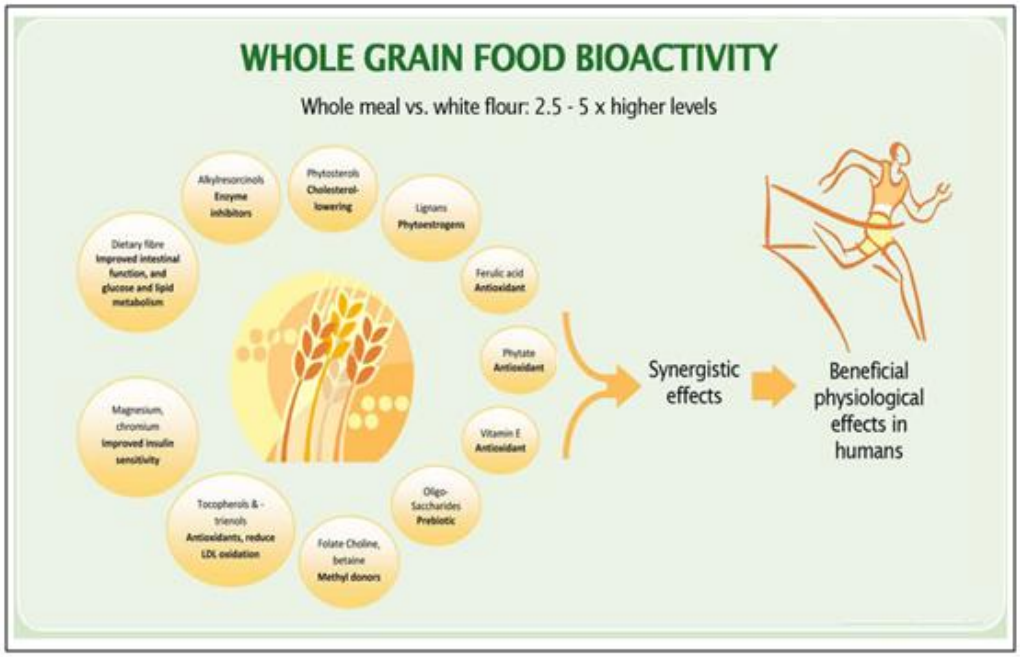
More recent evidences also indicate that cereal whole grains contain significant quantities of phytochemicals which may significantly contribute to reported health benefits of whole grain consumption (Ward *et al*., 2008).

These include: vitamins (B and E group and folate), minerals (selenium, iron, zinc and magnesium), **fiber** (lignans, β-glucan, soluble pentosans, **arabinoxylans**), phytosterols, sphingolipids, **polyphenols** and phenolics (hydroxycinnamic, ferulic, vanillic p-coumaric acids and **alkylresorcinols**), carotenoids (α- and β-carotene, lutein and zeaxanthin) and phytate (Figure 5).

Recent studies have identified many differences in the metabolic profiles of rats fed with whole and refined wheat grain. However, the components in whole grain that are responsible for these effects on the protection of health and homeostasis and their mechanism(s) of action are still not fully understood.

In fact, it is probable that several factors are involved and act additively or synergistically to achieve the favorable and advantageous effects (Bjorcka *et. al.*, 2012).

Figure 5 Bioactive compounds in whole grain (from "The Grains & Legumes Health Report").



1.2.1 Dietary fiber (TDF)

Among the bioactive compounds that are contained in the outer part of the kernel or in the germ, and, generally, removed during milling process for the production of refined flours (Ward, 2008), there are the **dietary fiber components**.
In all higher plants, elongated fiber cells constitute the structural elements, giving strength and shape to the tissue of stems, branches, and roots, but also to softer tissues such as leaves and flower.
TDF comprises mainly complex carbohydrates which are resistant to digestion and absorption in the small intestine although some of them could be at least partly used as substrates for micro-organisms in the large intestine (AACC, 2001 and 2003).
In fact, dietary fiber can be defined as:

"*the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated substances and promotes beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation*" (Andersson *et al.*, 1993).

Roberto Ciccoritti

This definition refers not only to the natural plant cell components but also to food additives with physiological effects, all being accepted as contributing to the benefits of dietary fiber (AACC 2001 and 2003).
The last definition of dietary fiber was written in 2006 by FAO/WHO Codex Alimentarius (CAC) based on physiological effects together with a classification of the structural components. In particular this definition clarifies that the term dietary fiber means:

"*carbohydrate polymers with a degree of polymerization not lower than three, which are neither digested or absorbed in the small intestine*".

Belonging to the group of fibers are: **arabinoxylans**, glucans, resistant starch, cellulose, lignans, pectins, glucomannans, lignins, galactomannans, insoluble pentosans, hemicellulose, soluble pentosans, oligosaccharides, inulin, fructoligosaccharides (FOS). In relation to different water solubility and capacity to form viscous gel it is possible to classify the fiber in two main groups water extractable and unextractable or soluble and insoluble dietary fiber to which different physiological effects have been ascribed in human (Saulnier *et al.*, 2007). The systemic and epidemiological impacts of these effects, however, are also associated with the presence of other compounds and not only with fiber (Manning and Gibson, 2004, Kabel *et al*., 2002;). Fiber slows down the passage of food in the upper intestinal tract but increases the rate of transit in the large intestine. The overall transit time is 3 folds reduced with a fiber rich diet respect to low fiber diets.

1.2.1.1 Arabinoxylans (AX)

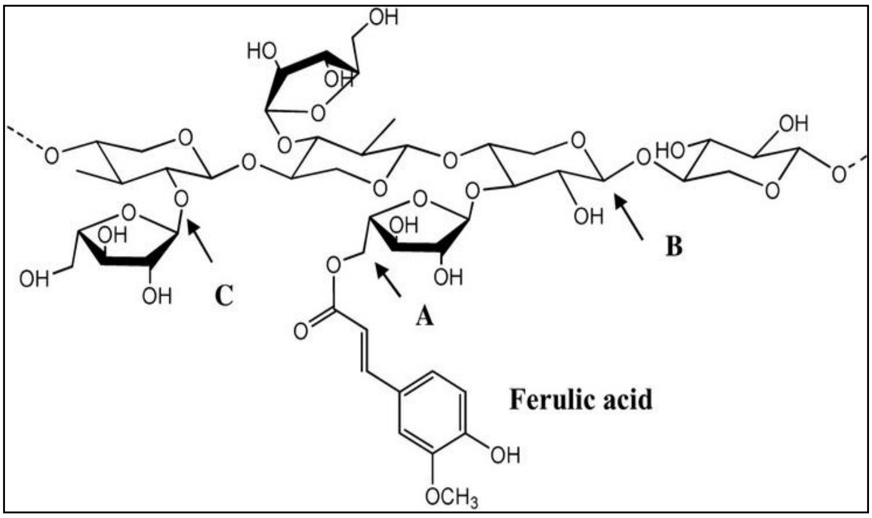
Arabinoxylan (AX) is one of the most important dietary fiber component in cereal grains (Gebruers *et al*., 2008; Andersson *et al*., 1993) and of particular interest for its positive effects on health. AX consist of a backbone of β-1,4-linked D-xylopyranosil residues substituted at the O-2 and/or O-3 position with α–L-Arabinofuranosyl residues (Fausch *et al*., 1963). Depending on the source and tissue, small levels of other substituents and side chain might occur.
Arabinofuranosyl residues can be substituted at O-5 with ferulic acid. The later can form cross-links by formation of di-ferulic acid bridges (Saulnier *et al*., 1995). AX are classified as water extractable (WEAX) and water unextractable (WUAX)

fractions, hence, an important source of both soluble and insoluble dietary fiber. Water extractability depends on the structural features of the polymer chain (degree of arabinose substitution, ferulic acid cross linking and degree of xylan polymerization) and, also, on covalent linkage to other cell-wall polymers (Courtin, 1998) (Figure 6).

AX, a heterogeneous and complex molecule, appears to play the major effect both on technological and nutritional properties of wheat through its high water-holding capacity and its ability to form highly viscous water solutions (Courtin *et al.*, 2002, Garcia *et al.*, 2007; Shewry *et al.*, 2009).

Both forms (WE-WU AX) affect flour functionality during cereal processing and/or the quality of end products in different way.

Figure 6 Structure of ferulic acid esterified to arabinoxylan, the main component of hemicellulose in monocots. (A) ferulic acid linked to O-5 of arabinose chain of arabinoxylan. (B) β-1,4-linked xylan backbone. (C) α-1,2-linked L-arabinose.



The physical properties of AX determine its physiological effects on the upper and lower gastro-intestinal tract. Fermentation of AX requires the presence of AX degrading enzymes. Intestinal species such as *lactobacillus*, *bacteroides*, and non-pathogenic *clostridia*, are specialized in degrading complex carbohydrates (Grootaert *et al.*, 2007). Species belonging to the *Bacteroides fragilis* group in particular play an important role in carbohydrate metabolism, due to their ability to produce a wide range of depolymerizing enzymes (Van Laere *et al.*, 2000). The rate, site and extent of fermentation is dependent on different fractions such as the degree of solubility,

the source and the chemical structure of the fiber, the concomitant availability of more readily fermentable fiber, the type and volume of colonic micro-flora, and the intestinal transit time. The main end products of fermentation are the short-chain fatty acids (SCFAs), butyric, propionic, and acetic acid and the gases, carbon dioxide, hydrogen, and methane (Anderson and Hanna, 1999). Short-chain fatty acids enhance immune protection by promoting the production of T-helper cells, antibodies, leukocytes, and cytokines, stimulate lymph mechanisms and stabilize blood glucose levels through their action on pancreatic insulin release. The fermentation also lowers pH and increases the absorption of dietary minerals (Anderson and Hanna, 1999, Wong *et al*., 2006). The low pH also protects against the formation of colonic polyps, inhibits inflammatory and adhesion irritants, and improves the barrier properties of the colonic mucosal layer, by contributing to large bowel immunity. Locally, SCFAs also stimulate the growth of beneficial bacteria like *bifidobacteria* and *lactobacilli* while inhibiting the growth of pathogenic species like *clostridium perfringens* and limiting the growth of carcinogen-producing bacteria (Craeyveld, 2009, Lopez *et al*., 1999). The lowered pH reduces the conversion of conjugated bile salts in the colon to secondary bile acids thus reducing the exposure of the large bowel mucosa to the potentially carcinogenic effects of the bile acids (Saulnier *et al.,* 1995).

Apart from its nutritional relevance, AXs are also important during cereal technological processes (Perlin, 1951). The role of arabinoxylans in the formation and properties of wheat flour dough has been the subject of many investigations. Wheat AXs have been shown to have significant influence on the water distribution and rheological properties of flour dough, starch retrogradation, and bread quality (Labat *et al.*, 2002). It is known that, in spite of their low concentration, the arabinoxylans are present in of wheat endosperm cell walls and contribute to the gluten protein interactions, but the exact role of AX in wheat flour gluten-starch separation is not yet clear. Addition of WEAX with different molecular weight (MW) to wheat flour during gluten-starch separation indicated the importance of the MW of these AX for their effect on gluten agglomeration. WEAX of low MW hardly affected the distribution of gluten proteins over the different sieves whereas WEAX of medium and high MW negatively influenced the formation of large gluten

aggregates. WEAX of high MW had a detrimental impact on the agglomeration behaviour (Frederix, 2004).

Since AXs have a high water binding capacity, these components are expected to have an important impact on pasta dough properties, during dough formation and pasta making a significant amount of AX is solubilized (Sisson, 2008).

1.2.2 Phenolic compounds

The phenolic compounds found in wheat grain are various but have common features of the basically phenols, i.e. molecules containing one aromatic ring: phenolic acids, (such as ferulic acid, sinapic acid, p-coumaric acid, etc.) **5-n-alkylresorcinols,** and vitamin E (Table 1). Phenolic compounds display antioxidant activity by different multi-faceted antioxidant mechanisms. Their free radical scavenging activity is well documented. The hydroxyl group of the phenolic ring donates one electron to the radical molecule, which is followed by a rapid proton transfer. The result is the transfer of a hydrogen atom to the free radical and so the phenolic oxidation. However, the phenol radical does not progress the oxidative reaction, since it is relatively stable due to resonance structure, in which the unpaired electron is delocalized to the ortho or para position of the phenyl ring.

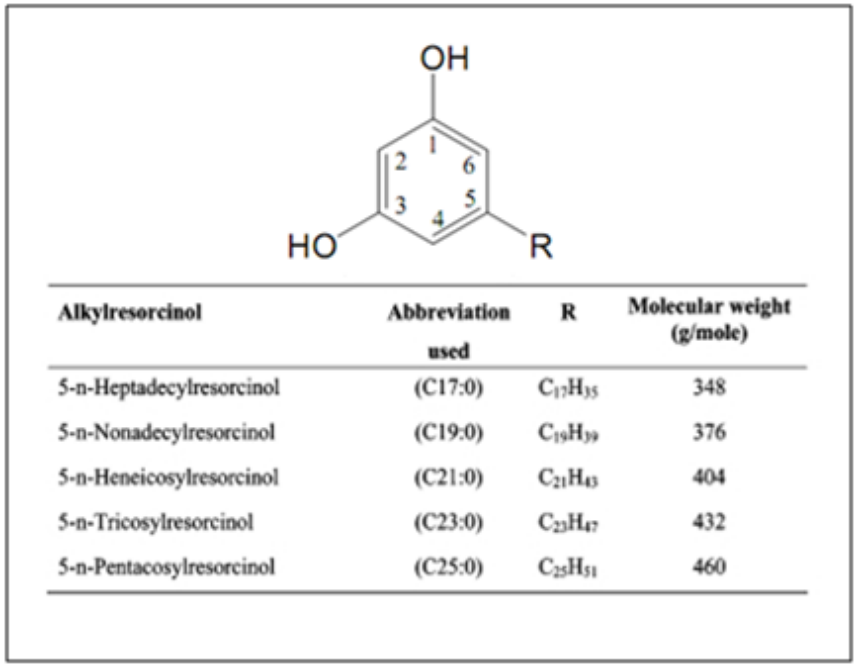
Finally, the oxidized antioxidant can be converted back to its reduced form by enzymatic and non-enzymatic antioxidants.

1.2.2.1 5-n-alkylresorcinols (ARs)

Alkylresorcinols (ARs) are members of a family of compounds referred to as phenolic lipids, which have been identified in numerous plants, fungi and bacteria, but relatively few animal species. Among the major classes of phenolic lipids the ARs are biosynthesized in plants in different forms. New insights from an ancient enzyme family include alkylphenols, alkylresorcinols, anacardic acids and alkylcatechols, but the alkylresorcinols are by far the most prevalent in nature (Moore *et al*., 2007). In higher plants, alkylresorcinols typically occur as mixtures of homologues possessing side chains of 13 to 27 carbons with varying degrees of saturation. Fungi and bacteria similarly accumulate mixtures of alkylresorcinols with different chain lengths, however microbial homologues all possess saturated side chains (Slavin *et al*., 2004). In cereal species specific ARs called 5-n-alkylresorcinols

are found. Their structure is characterized by two hydroxyl groups in position C1 and C3 of the aromatic ring and a saturated alkyl chain in position C5, with a length that can vary from 17 to 25 carbon atoms (Ross *et al*., 2003) (Figure 7).

Figure 7 Structure of 5-n-alkylresorcinol commonly found in cereals (from Ross et al 2011).



Alkylresorcinol	Abbreviation used	R	Molecular weight (g/mole)
5-n-Heptadecylresorcinol	(C17:0)	$C_{17}H_{35}$	348
5-n-Nonadecylresorcinol	(C19:0)	$C_{19}H_{39}$	376
5-n-Heneicosylresorcinol	(C21:0)	$C_{21}H_{43}$	404
5-n-Tricosylresorcinol	(C23:0)	$C_{23}H_{47}$	432
5-n-Pentacosylresorcinol	(C25:0)	$C_{25}H_{51}$	460

Cereal alkylresorcinols are predominantly present as saturated derivatives; however, unsaturated, keto and hydroxy derivatives have also been found, especially in rye (Ross *et al*., 2004; Kozubek and Tyman, 1999).

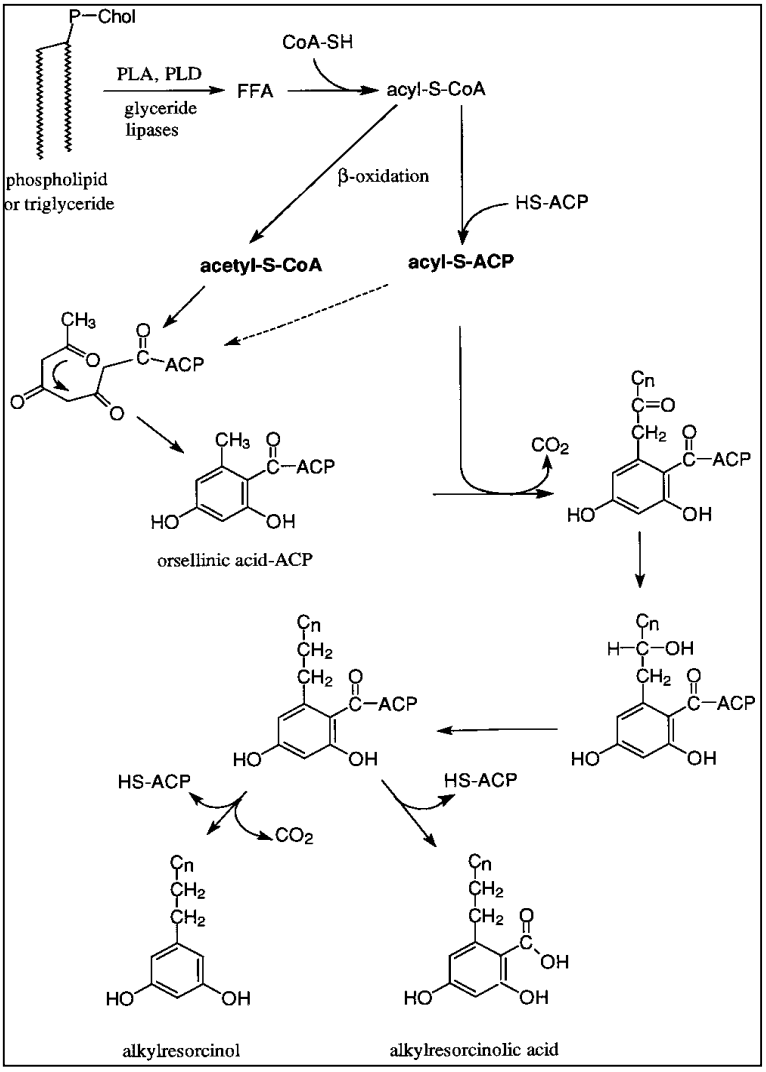
The trivial names of the different AR derivatives indicate, similar to fatty acids, the chain length and the degree saturations (e.g., C17:0).

In cereal kernels they are not evenly distributed, but there is a concentration gradient with higher values in the pericarp, intermediate values in the aleurone layer and very small concentrations in the endosperm fraction of the seed (Landberg *et al*., 2009). The amphipathic nature of their structure (characterized by one hydrophobic and one hydrophilic domain) seems to be responsible for their ability to interact with the biological membranes, by altering their properties, but also with enzymes and proteins, by modifying their activity (Kozubek and Tyman, 1999; Ross *et al*., 2003). The biological effects of alkylresorcinols are weak antioxidant capacity, high ability

Roberto Ciccoritti

to stabilize biological membranes, antimutagenic properties and anticancer, antifungal and antibacterial properties; the ARs also seems to inhibit the glycerol-3-phosphate dehydrogenase and thus prevent the accumulation of triglycerides in the adipocytes (Kamal-Eldin *et al*., 2000; Rejman and Kozubek, 2004). The potential antimicrobial activity demonstrated for many naturally occurring alkylresorcinols and alkylresorcinol derivatives by *in vitro* assays may be in part responsible for their defensive role in plants (Zarnowski *et al.*, 2002; Moore *et al*., 2007; Curtis *et al*., 2002; Nocente *et al*., 2012). In fact, the highly localized pattern of deposition within regions surrounding plant structures coupled with their demonstrated antimicrobial activity could indicate a role for alkylresorcinols in the formation of defensive chemical barriers. For example, significant accumulation of ARs occurs within grains of cereals such as wheat, rye, triticale and barley, existing within a thin cuticle layer external to the seed coat. 5-n alkylresorcinols have also been found to be concentrated within the cuticles of rye leaves, with similar homologue compositions occurring on the adaxial and abaxial leaf surfaces (Baerson *et al.*, 2010). Root systems of *Oryza* spp. exude an alkylresorcinol mixture, and likewise, *Sorghum* spp. exudates contain the alkylresorcinol derivative sorgoleone, which is an allelochemical possessing antifungal activity. The amphiphilic (surfactant-like) characteristics of alkylresorcinols and derivatives such as sorgoleone could promote the formation of thin exudate layers completely covering root systems, thus providing a continuous defensive boundary. This situation could be analogous to that proposed for the leaf trichome exudates of *Lycopersicon* spp., where the formation of continuous antifungal chemical barriers may be facilitated by the presence of amphiphilic acylglucose exudate constituents. The AR concentrations range from about 100 to 1500 μg/g d.m. (dry matter) for wheat and rye, in relation to the cultivar and the growing environment, while in barley remain in the order of some 10 to 50 μg/g d.m. (Ross *et al*., 2003). ARs are synthesized, through a cycle of biosynthetic polyketides from fatty acids, by condensation with three molecules of malonyl coenzyme A (malonyl-CoA). Subsequent steps leading to the formation of the acid 6-alkylresorcinol from which, by decarboxylation, will form the 5- alkylresorcinols (Figure 8) (Suzuki *et al.*, 2003).

Figure 8 Scheme of alkylresorcinol biosynthesis (from Suzuki et al., 2003).



1.3 Intrinsic and extrinsic factors affecting cereal whole grain composition

1.3.1 Genetic and environmental influence on Arabinoxilan (AX), Alkylresorcinol (ARs) and phenolic compound contents of wheat whole grain

1.3.1.1 AX variability

Numerous studies investigated the influence of genotype and growing environment on the AX contents of the wheat grain (*T. aestivum*). For total AX, a range between 5.5 and 7.8% (d.m.) was referred for wheat grain by Saulnier *et al.* (1995). Large variations in content and structural features of WEAX in wheat flour

Roberto Ciccoritti

of French varieties and the large impact of genotype on AX structure were evidenced by Ordaz-Ortiz and Saulnier (2005) and Saulnier *et al.*(1995). Genetic control of AX structural variability and genetic differences among genotypes for arabinoxylan fractions were also indicated (Hong *et al*., 1989; Li *et al*., 2009, Martinant *et al.*, 1999). Moreover, Finnie *et al*. (2006) showed in soft wheat that the variations in AX content were primarily due to genotype, while the environment had a secondary effect. Few studies on the AX in durum wheat grains are available. Total arabinoxylan contents between 4–6% (d.m.) were indicated by Lempereur *et al*. (1997) by analyzing the whole grain of five French durum wheat varieties and by Gebruers *et al*. (2008) by examining a set of European durum wheat varieties within the HEALTHGRAIN project. Turner *et al*. (2008) found a wider range of water extractable arabinopolymer contents (0.59–7.21 μg/mg) in Australian durum genotypes than in bread wheat cultivars and indicated their positive influence on pasta quality through a significant reduction of pasta stickiness.

1.3.1.2 Phenolic compound and AR variability

The content of phenolic compounds is affected by several factors such as plant species, cultivar, organ, physiological stage and environment (soil, agronomy and climate) (Carbone *et al.*, 2011; Scalbert and Williamson, 2000; Yu *et al*., 2004).
Rye grain, is the species with the highest AR content ranging between 500 to 1300 μg/g d.m. (Nystrom et al., 2008; Ross, 2012), followed by whole wheat grain considered one of the main dietary sources of ARs, with contents ranging from 399 (mean value of ten durum wheat varieties) to 605 μg/g d.m. in five varieties of spelt (Andersson et al., 2008). Recently, Menga *et al.* (2010) reported that location had the greatest impact on the total polyphenolic content (TPC) of a set of durum wheat varieties while Yu et al. (2004) referred significant effects both of variety and growing location on antioxidant properties and TPC of hard winter wheat. Mpofu *et al*. (2006) observed a greater impact of environment (E) than of genotype (G) on the antioxidant activity and phenolic content of hard winter wheat, with G × E interactions scarcely significant. Andersson *et al.* (2010) and Shewry *et al.* (2010) studied the effects of G and E on the AR content of winter and spring wheat grain. Significant variations were observed among different locations, years and varieties, suggesting that genetic and environmental factors could affect AR accumulation.

Recently Gunenc *et al*. (2013) confirmed these results by highlighting significant effects of region and wheat cultivars and their interactions on AR bran contents.

1.3.2 Technological process influence on nutritional value of cereal whole grain products

1.3.2.1 Milling process

The milling process of cereal grains is known to induce compositional and nutritional changes in cereal products. In traditional technological conditions, milling process is considered critical for the nutritional value of cereal flours and this is due to the existence of the unique bioactive compounds of whole grain. Following the recommendations of the guidelines and the new trend for the consumption of foods functional for human health, whole grains can strongly contribute to increase the consumers intake of fiber and associated bioactive compounds, their importance in nutrition and health being well defined (Ward *et al*., 2008; Saura-Calixto *et al*., 2009). Besides, many investigations on Mediterranean diet confirmed the link between this appropriate dietary pattern and the prevention of chronic diseases. Trichopoulou and Lagiou, (1997) reported nine main components in the definition of Mediterranean diet, among them it was recommended "high consumption of cereals, mainly unrefined cereals and bread" (Saura-Calixto and Goni, 2009). Pasta and bakery products are the traditional wheat-based products generally obtained with refined grains, i.e. by removing the outer part of the kernel during milling. In this way the traditional wheat products present a low bioactive component content. In fact, as previously discussed, most bioactive components (vitamins, minerals, antioxidants, phytoestrogens and fiber, soluble and insoluble) are contained in the outer parts of the kernel or in the germ, that are removed during the traditional milling process (Camire *et al*., 2004). As well known, the objective of the traditional milling system for cereal grains such as wheat is the separation of the endosperm from the various outer layers and the elimination of the germ. Following the new trend for healthy food production, cereal whole grains have received much attention, especially in order to minimize losses of healthy constituents of grains during processing (Liu, 2007; Shewry, 2009; Jones *et al.* 2010; Slavin, 2003). Therefore innovative technologies have been developed at level of grain milling to produce flours richer of the outer layers by ensuring food safety requirements (Michalska *et*

al., 2007; Hemery *et al*., 2007; Ferrari *et al*., 2009; Delcour *et al*., 2012). These studies described different processes for the production of nutritionally enhanced ingredients and products. Particular attention was focused on the impact of grains pretreatments and bran fractionation as well as to preprocessing prior to milling as degermination, debranning, histological fractionation, in this last case, different separation methods, such as size-classification, air-classification, or electrostatic separation, can be combined to obtain efficient separation of the tissues.

1.3.2.2 Hydrothermal process

In the development of new whole grain foods different processes based on heat treatment were examined to identify the best conditions suitable to protect or improve the technological and nutritional properties of cereal whole grain (Ogbonnaya *et al*., 2009). Important changes in physical, chemical and sensorial characteristics of rice seeds occur during parboiling, some of them associated with nutritional advantages (Heinemann, 2005). Hydrothermal process as parboiling has been also applied to other cereals. Young *et al*. (1993) explored the effects of parboiling process on sorghum starch characteristics, while the results of Hidalgo *et al*. (2008) showed that the nutritional value of parboiled einkorn *(Triticum monococcum*) kernels improved in comparison with untreated seeds due to partial shift of some nutritional compounds from the outer layers to the inner ones. Unique chemical, physical and structural properties were observed in the numerous studies on isolated oat starch, data showed that specific structural features (Subaric *et al*., 2011) could be in strong relationship with the high lipid content (Hartunian *et al*., 1992; Wang and White, 1994; Zhou *et al*., 1998). On the other hand, considerable differences were observed in swelling properties and amylose solubility of oat starch in comparison with other cereals.

In this context, it was observed that the use of steam in the parboiling process improves the oat technological characteristics useful for pasta production (Redaelli *et al.*, 2006).

Furthermore, the appropriate parboiling conditions (e.g. temperature and humidity) could emphasize the nutritional grain properties. In fact, during the heat treatment the starch gelatinization facilitates the formation of resistant starch, a type of dietary fiber, associated with several health benefits (Kim *et al.*, 2006). Moreover the

parboiling process promotes the migration of lutein and tocols (vitamin E) from the embryo to the starchy endosperm, increasing the nutritional meal value (Hidalgo *et al.*, 2008). Finally, the parboiled grains present a good firmness, and taste that allow the direct consumption, as soups and risotto. The parboiling process present several advantages: (i) diffusion of vitamins and minerals from the outer kernel layers into the inner part of the grain, (ii) enzyme inactivation, (iii) prevention of fungi and insects proliferation, (iv) gelatinization of starch granules with positive effects on kernel hardness and on the broken kernels percentage, (v) increase in milling yield.

Roberto Ciccoritti

2 Aims

Numerous epidemiological studies have shown that the consumption of whole grain foods could allow an adequate intake of bioactive compounds useful in reducing risks of several diseases. Whole cereals grain contains various bioactive compounds beneficial to the human health, including mainly dietary fibers and antioxidant substances.

The general objective of this thesis is to allow a better knowledge of main intrinsic and extrinsic factors influencing the production of whole grain based products nutritionally improved for increased levels of dietary fiber and/or antioxidant compounds.

To achieve this aim, the main objectives of this thesis were:

- to investigate the AX content in a wide range of Italian durum wheat cultivars, evaluating the effects of genetic and environmental factors on the final AX level as well as on its extractability in whole durum wheat grains;
- to analyze the AR content of different durum wheat cultivars and to relate them to the total soluble phenols and antiradical activity (AA). Moreover, a particular emphasis has been devoted to investigate the effect of genotype (G) and environment (E) factors and G x E interactions on the examined durum wheat properties;
- to explore the extent of variation caused by genetic factors on AR and STP phytochemicals determining their amount and chemical composition as well as the total antiradical activity of grain by comparing ancient (*T. monococcum*, *T. turgidum ssp dicoccum* and *T. turgidum ssp. turanicum*) and modern (*T. turgidum ssp durum* and *T. aestivum*) *Triticum* species;
- to investigate the influence of milling and hydrothermal pretreatment on the potential nutritional quality of the durum wheat raw material for whole grain based food production.

Roberto Ciccoritti

3 Material and experimental plan

The content of this thesis is the result of more approved research projects. For this reason it is preferred that the different materials utilized as well as the experimental plan applied are described in detail in the first part of the each section of results. The following section describes only the analytical methods common to all projects.

4 Analytical methods

4.1 Physical analyses

4.1.1 Sample pretreatment

Immediately after harvest, all the grain samples used in this study were milled using a laboratory cyclone mill (Cyclotec 1093, Foss, Italy) to pass a 0.5 mm screen, to produce wholemeal which was stored at 4° C.

The wholemeal was thoroughly mixed to ensure uniformity and all analyses were carried out in triplicate on two independent aliquots of each sample.

4.1.2 Moisture determination

Moisture content was determined at 120° C with a thermo balance (Sartorius MA 40, Gottingen, Germany). All values are reported on a dry matter basis.

4.1.3 Grain physical characteristics

Single Kernel Classification System (SKCS 4100, Perten Istruments Sweden) was utilized for the determination of grain hardness (expressed as hardness index), diameter (mm) and 1000 kernel weight (g).

4.2 Chemical products and analyses

All reagents were of analytical grade (Carlo Erba, Roma, Italia). D(−)arabinose, D(+)xylose, D(+)mannose, D(+)glucose monohydrate were purchased to Fluka; D-(+)galactose (Sigma Aldrich) and internal standard β-D-allose (Acros Organics or Alfa Aesar). Folin-Ciocalteu reagent, pyridine and all organic solvents were purchased from Carlo Erba (Italia). (+) catechin, 1,1-diphenyl-2-

Roberto Ciccoritti

picrylhydrazyl radical (DPPH•), Fast blue B salt and methyl behenate (C22:0, methyl docosanoate) were purchased from Sigma–Aldrich (Milan, Italy).
Trimethylchlorosilane (TMCS), which was used to prepare the trimethylsilyl ether derivates of AR extracts, was purchased from Chebios (Roma, Italia). Standards 5-n-pentadecylresorcinol (C15:0), 5-n-heptadecylresorcinol (C17:0), 5-n-nonadecylresorcinol (C19:0), 5-n-heneicosylresorcinol (C21:0), 5-n-tricosylresorcinol (C23:0) and 5-n-pentacosylresorcinol (C25:0) were purchased from ReseaLife Chem. Science (Burgdorf, Switzerland).

4.2.1 Protein

The protein content was estimated with the nitrogen combustion method (Dumas) using the Leco-FP 528 nitrogen analyzer (AACC 1995).

4.2.2 Arabinoxylans (AX)

4.2.2.1 Gas chromatographyc-flame ionization detector (GC-FID) analysis

The arabinoxylan determination as total (TOAX) and unextractable (WUAX) was performed with gas chromatography (GC-FID). The cell wall material (CWM) was isolated according to the procedure of total (TDF) and insoluble (IDF) dietary fiber (Lai *et al.,* 2007 and AOAC 1995) by using an enzymatic kit for fiber determination (Bioquant, Merck, Germany). Briefly, 0.5 g of milled grains were incubated in 0.08 M phosphate buffer at pH 6.00 with heat-stable α-amylase at 95–100° C for 30 min, followed by cooling and hydrolysis with protease at 60° C for 30 min at pH 7.5. Successively, at the same temperature, the last enzymatic step with amyloglucosidase at pH 4.5 was performed. For TOAX analysis, the polysaccharide material was further precipitated with 95% ethanol (T = 60° C) and then filtrated with the Fibertec semiautomatic system (FossItalia); this procedure found the CWM to contain 2.4–2.8% of proteins. The residue for WUAX determination was isolated by directly filtrating after the treatment with amyloglucosidase. Both residues were dried in oven at T = 105° C overnight, and, then, quantitatively recovered and hydrolyzed with 10 mL of 2M TFA at T = 121° C for two hours in a screw-capped glass tubes.

Roberto Ciccoritti

Total neutral sugars were estimated by GC-FID (Albersheim *et al.,* 1967; Englyst *et al.,* 1994), by using the following standard sugar mixture: D(−)arabinose, D(+)xylose, D(+)mannose, D(+)glucose monohydrate (purity more than 99%), D-(+)galactose (purity more than 99%) and internal standard β-D-allose (purity 99%). The following chromatographic conditions were employed: column temperature 220°C; injection and detector temperature 275°C, helium flow rate 1.5 mL min−1. The instrument (Clarus 600, PerkinElmer, Shelton, USA) was equipped with an autoinjector and a Restek capillary column (RTX 2330 30 m, 0.32 mm, 0.2 μm).

The AX and WUAX contents were calculated as in Ingelbrecht *et al.,*(2000) (eq:1):

(eq:1) AX = [%xylose + %arabinose − (0.7 × %galactose)] × 0.88.

The soluble AX (WEAX) was calculated by difference: TOAX–WUAX.

4.2.2.2 Fast colorimetric method analysis

The colorimetric method of Douglas (1981) was also used to determinate the total AX content (expressed as percentage of xylose). The method was modified as follows: flour (10 mg) was weighed into a stoppered bottle adding 4 mL of distilled water and 20 ml of the freshly prepared extracting solution (110 mL acetic acid, glacial; 2 mL concentrated hydrochloric acid, 20% w/v in ethanol 5 mL phloroglucinol; 1.75% w/v aqueous 1 mL glucose) were added. The bottles were placed in a boiling water bath for 25 min and shaken twice during the incubation. The percentage of xylose in the flour was calculated by subtraction of the reading at 510 nm from that at 552 nm (absorbance 510nm – absorbance 552nm) and by comparing the results with a standard curve obtained using a stock solution of 10 mg of xylose in 100 ml of water (eq2). Aliquots of 0.5, 1.0, 1.5, 2.0 mL of stock solution were made up to 4.0 mL with distilled water and treated as previously described for the samples. For the determination of water-extractable arabinoxylans the method described by Finnie *et al.,* (2006) with the indications of Douglas (1981) was performed. Briefly, 125 mg of flour was placed in a tube and 25ml of distillated water were added. The suspension was extracted by stirring for 30 min and then centrifuged at 2500 rpm; supernatant 1ml was removed and placed in a stoppered

bottle for the determination of water-extractable arabinoxylans. Water-Extractable arabinoxylan content was calculated using the equation (eq.2) provided by Finnie *et al.*, (2006).

(eq2): Arabinoxylan content (mg/g)=1,000x[($AA_{552-510}$ sample)x{(xylose equivalent, mg)/($AA_{552-510}$ standard)}+(xylose equivalent intercept, mg)

4.2.3 Alkylresorcinols (ARs), Soluble Total Phenols (STPC) and Antiradical Activity (AA)

4.2.3.1 Extract preparation

Extracts for the determination of ARs, STPC and AA were prepared as follow. 1.0 g of wholemeal was placed in a 50-mL tube and subjected at continuous mechanical shaking for 48 h at room temperature with 40 mL acetone. The extracts were then filtered through Whatman No 42 paper and evaporated to dryness at 60° C in a rotary evaporator (Buchi R-114, Switzerland). The dry residues from all the extractions were then dissolved in pure methanol (1 mL) and immediately analyzed for AR and STP content and antiradical activity (AA).

4.2.3.2 Determination of AR content: Fast method

AR extracts were also analyzed by a fast colorimetric method based on the use of Fast Blue B salt, according to Gajda *et al.* (2008). The total AR content was calculated by a calibration curve, using 5-n-pentadecylresorcinol (C15:0) as standard. Results are expressed as μg/g of whole milled grain (d.m.).

4.2.3.3 Determination of STPC

The STPC content was determined using the Folin–Ciocalteu (F–C) method as reported by Moore and Yu, (2008). STPC was calculated by a calibration curve, using (+) catechin as a standard. Results are expressed as micrograms of catechin equivalents (CTE) per g of whole milled grain (d.m.).

4.2.3.4 Determination of AA

The di(phenyl)-(2,4,6-trinitrophenyl) iminoazanium (DPPH•) quenching capacity of wholemeal extracts was estimated by spectrophotometric procedure as

reported by Carbone *et al.* (2011). AA (EC50) was calculated according to Sanchez-Moreno *et al.* (1998), using a calibration curve obtained with different amounts of methanolic extracts obtained following the procedure of Gaida *et al. (*2008). Results are expressed as EC50 = mg of wheat milled grain (on d.m. basis) required to obtain 50% DPPH scavenging.

4.2.4 Determination of ARs with GC–MS

4.2.4.1 Extract preparation

For determination of AR relative homologue composition by GC-MS, wholegrain (1.0 g) was placed in 50-mL tubes and extracted by continuous shaking at room temperature with 40 mL acetone containing 200 µL of 1 mg/mL w/v methyl behenate (internal standard) for 24 h. The extracts were then filtered through Whatman No 42 paper and analysed by GC-MS.

4.2.4.2 GC–MS analysis

Total AR content in whole grain extracts was determined by GC–MS, according to Landberg *et al*. (2009) using methyl behenate as internal standard (IS). Briefly AR extracts (10 mL) were dried under nitrogen and silylated with a mixture (400 mL) of pyridine and trimethylchlorosilane (TMCS; 9:1 v/v). The mixture was then shaken and heated at 70° C for 60 min. GC–MS analysis was performed with a Perkin Elmer gas chromatograph GC Clarus 600 series coupled to a mass spectrometer Clarus 580D (Perkin Elmer, Milan, Italy) equipped with a split/splitless injector, a RTX-5MS column (0.25 mm 30 m, 0.25 µm film thickness, Restek, Milan, Italy) and a quadrupole mass spectrometer (Clarus 580D, Perkin Elmer, Milan, Italy) operating in electronic ionization(EI) (70 eV). Helium was used as the carrier gas (1 mL min−1). The temperature program was as follows: 250 °C (0 min),to 320 °C (20.0 min), 320 °C (20.1 min), to 330 °C (10 min). The ion source and detector temperatures were 250 and 350 °C, respectively. The injector temperature was 325 °C. The total ion current (TIC) mode was used to record the positive ion mass spectra of the samples in the range between m/z 50 and 650. The AR contents were determined by comparing the relative retention times with those obtained for a mix of AR homologue standards:C15:0, C17:0, C19:0, C21:0, C23:0, C25:0. The AR content was obtained by summing the amount of each homologues.

4.4 Technological processes

4.4.1 Traditional milling

Durum wheat grains (3-5kg) were cleaned, washed and conditioned to a water content of 16%; the washed grains were left moistened overnight. Standard milling was performed by a pilot plant with three breaking and three sizing rolls (Bühler MLU 202) in order to obtain semolina, traditional raw material for pasta production (Italian Law No. 580, 1967 and following modification 2001).

4.4.2 Micronization and air classification process

The Micronization KMX-500 device, 100-200 kg/h (Separ Microsystem, Brescia, Italy), that consists of a steel drum containing a rotor operating at various peripheral speeds; two peripheral speeds were used for wheat grains (as is and parboiled): 85 and 170 Hz. The obtained whole flours were then fractioned by air classification with an Unit Integrated Turbo separator air (Separ Microsystem, Brescia, Italy). The air classification system sorted the flour into three principal fractions: two fine fraction F1 and F2 and coarse fractions G1.

4.4.3 Hydrothermal process

The hydrothermal process was carried out as described below.
Soaking Conditions: 100 g of each samples were directly soaked with 50 ml of water at room temperature for 240 min (Hidalgo *et al.*, 2008)
Steaming Conditions: the samples were treated in autoclave 121°C for 10 min to increase sample moisture to 30-35% (d.m.) (Kimura *et al.*, 1976).
Drying Conditions: Drying step was carried out at 30±1° C for 72 hours in the oven. Then the samples were vacuum stored.

4.5 Statistical analysis

Analysis of variance (ANOVA) was performed with the MSTATC program (Michigan State University, East Lausing, MI). Principal Component Analysis (PCA) was performed with MATLAB software (R2010a version, MathWorks Inc., USA); finally, box-plots were used to display descriptive statistics (the median, the upper and lower quartiles and the minimum and maximum data values).

Roberto Ciccoritti

5 Results

5.1 Effects of genetic and environmental variations on specific bioactive components (TOAX, WEAX, ARs, STPC and AA) of the durum wheat whole grain

5.1.1 Samples and experimental design

For this study, a group of thirty Italian commercial cultivars of durum wheat (*Triticum turgidum L.* var *durum*) was obtained from a set of agronomic trials carried out each year by the Consiglio per la Ricerca e sperimentazione in Agricoltura, Research Unit for Cereal Quality (CRA-QCE). The trials allowed to evaluate agronomical performance yield and quality of durum wheat varieties grown in different sites under normal agronomic conditions. In each experiment, all cultivar were sown in 10 m^2 plots in randomized blocks with three replications. Nitrogen fertilization was applied according to local practice (about 150 and 90 U/ha, at Jesi and Foggia, respectively), with previous crop of fallow and leguminous in the Foggia and Jesi trials, respectively.

For the comparison among different environments (4 environments obtained by combining year and location), a sub set of nineteen varieties was considered. All determinations were carried out in duplicate on two independent aliquots of each sample obtained blending the three yield replicates, the results were expressed as mean ± standard error. Analysis of variance (ANOVA) was performed using a factorial model (mod.9) with G, E (locality and year) and G × E interaction. Genotypes were classified using Duncan's multiple range test ($p \leq 0.05$) by combining the results across environments. Principal Component Analysis (PCA) was used to study the variations associated with the genotype and the environment. Pearson's correlation coefficients (r) were also calculated. Correspondence analysis was performed between AR, STPC and AA arbitrary category and environment variables. Box-plots were used for comparisons among varieties and environments.

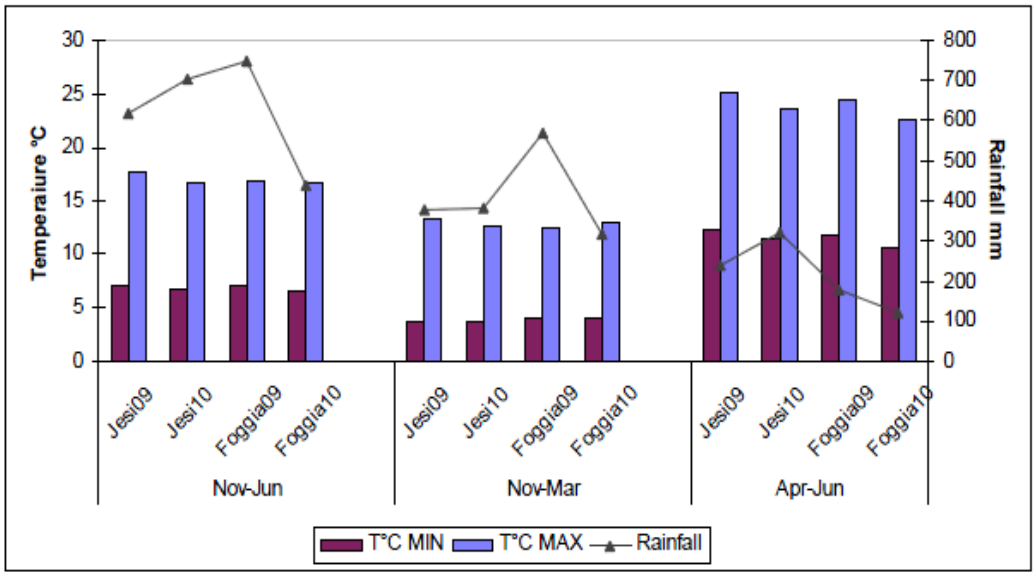
5.1.2 Environment description

Two Italian areas traditional for durum wheat cultivation (Jesi, Central-Northern Italy, and Foggia, Southern Italy) in two consecutive years (2008–2009 and 2009–2010) were considered. Jesi (altitude 97 m s.l.) is characterized by a continental climate with a cold-humid winter and sultry-humid summer. Foggia

(altitude 76 m. s.l.) is characterized by a Mediterranean climate, with warm summer and mild winter.

The mean temperatures and total rainfall, referred at three seasonal periods, are reported in Figure 9. The means of minimal temperatures for the three considered periods were similar for the two site and two crop years, whereas the maximum values of temperature presented differences from April to June between the two years, Foggia09 and Jesi09 having higher temperatures (mean values: 24.4 and 25.2°C, respectively) in comparison with the successive year (mean values: 22.6 and 23.6°C for Foggia10 and Jesi10, respectively). Anomalous precipitations occurred during the two trial years; moreover, total rainfall showed important differences between years and areas 616 and 749 mm at Jesi09 and Foggia09, respectively, and 702 for Jesi10 and 440 mm Foggia10.

Figure 9. Average temperatures (°C) and rainfall (mm) in the growing environments: Foggia09, Foggia10, Jesi09 and Jesi10 in three periods of durum wheat growth.



5.1.3 Grain characteristics and relationships among grain traits of the whole data set (30 durum wheat varieties).

Table 3 shows the mean values and the standard deviations of physical and chemical characteristics of the grains of thirty cultivars in each of the four

environments. On average, the environments presented variations for grain weight, whereas the other physical characteristics did not differ significantly.

Table 3. Grain physical and chemical characteristics (mean values on dry matter, and standard deviation) in thirty durum wheat varieties grown in four environments (locality and year).

Quality Traits	Environments			
	Foggia09	**Foggia10**	**Jesi09**	**Jesi10**
1000 Kernel Grain weight (g)	44.7±4.1	43.6±3.3	47.2±5.8	46.7±3.6
Grain diameter (mm)	2.7±0.1	2.7±01	2.8±0.2	2.8±0.1
Grain Hardness Index	82.7±4.6	88.0±5.1	88.7±3.7	86.8±3.6
Protein content %	12.10±0.82	12.11±0.64	16.2±0.79	14.7±1.46
TOAX %	4.70±0.33	4.60±0.27	4.51±0.31	4.81±0.41
WUAX %	4.01±0.29	4.01±0.31	3.91±0.29	4.22±0.44
WEAX %	0.72±0.31	0.61±0.17	0.61±0.23	0.63±0.29
ARs µg/g	240±39	310±54	252±43	253±45
STPC µg/g	130±19	105±22	123±32	130±19
AA *	109±28	124±28	128±21	153±27

*EC50 = mg of wheat milled grain (on d.m. basis) required to obtain 50% DPPH scavenging.

The hardness index, 1000 kernel weight and grain diameter values indicated that the durum wheat samples analyzed exhibit low variability for each of the considered environments. As expected, the protein concentration was highly sensitive to the growing site. In fact, durum wheat varieties grown in Jesi showed protein content greater than those grown in Foggia in both the years; these differences likely reflected the major availability of nitrogen fertilizer during grain filling and different soil fertility. The average TOAX content of the four environments ranged from 4.5 to 4.8% d.m., Jesi09 having lowest WUAX content than the other environments. As indicated in the experimental section the WEAX amount was calculated by difference (TOAX–WUAX); on average, among the four environments the WEAX range was very limited. In term of monosaccharide compositions WUAX appeared mainly composed by arabinose and xylose (mean values: 38.1% and 49.0%, respectively), small amounts of mannose were also found. WUAX had higher level

of arabinose and xylose if compared to TOAX. No significant differences in the AX composition were found among the durum wheat varieties (*data not reported*). The ratio WEAX/WUAX, considered a measure of extractability (Lempereur *et al*., 1997), varied from 10.9 to 23.4% with a mean value of 16.3%. As previously found by Lempereur *et al.* (1997), the extractability of AX in this study appeared to be independent from the TOAX content (*data not shown*). The AR contents ranged from 240 to 310 µg/g (d.m.) in Foggia09 and Foggia10 respectively. The average STPC values appeared independent from environmental conditions and the maximum was found in Foggia09 (about 130 µg/g d.m.). The AA values ranged between 109 and 152 EC50 (mg of wheat milled grain on d.m. basis required to obtain 50% DPPH scavenging).

Significant correlations between protein content and the grain physical characteristics were found:(r = 0.317 $p \leq 0.01$; r = 0.379 $p \leq 0.001$; r = 0.225 $p \leq 0.05$, with hardness index, grain weight and diameter, respectively, n = 76). The reported relationships were significant both in the single environments and in the whole trial. No correlations between AX fractions and hardness index were found, the data are in agreement with the result of Bettge and Morris (2000), who suggested a minimal role of pentosans in modifying hard wheat grain hardness, and with the results of Li *et al*., (2009) in hard and spring wheat.

Significant but low statistical relationships were found between the antiradical activity and soluble phenolic contents (r:−0.36; p <0.01) and between ARs and STPC (r:−0.18; p < 0.05), while no correlation among ARs, AX, AA and physical characteristics of the kernel was found

5.1.4 ANOVA in the 19 durum wheat cultivars common to the four environments.

To study the influence of genotype and environment, a group of 19 durum wheat cultivars common to the four environments was considered for ANOVA of AX, ARs, STPC and AA. The results presented in Table 4 underlined significant genetic and environmental effects and a significant genotype x environment interaction for all the parameter analyzed. However, as previously shown by Finnie *et al*., (2006) and by Gebruers *et al*., (2010) considering the fiber components in wheat wholemeal, the contribution of this interaction to the total variability was considerably lower than that of genotype or environment. The variety had the

greatest influence on the WUAX content in durum wheat samples and was important for TOAX.

Table 4. Mean Square of variety, environment and their interaction for arabinoxylan fraction content (total, TOAX, water unextractable, WUAX, and water extractable, WEAX), 5-n-alkylresorcinol (ARs), soluble total phenolic contents (STPC) and antiradical activity (AA) in the 19 durum wheat varieties grown in four environments.

Source of variation	D.F.	TOAX	WUAX	WEAX	ARs	STPC	AA
Varieties (G)	18	749.1 ***	994.8***	94.8***	8674.2***	1440.5***	1515.5***
Error	18	0.001	0.001	0.001	205.5	123.8	381.1
Environments (E)	3	781.96***	530.05***	120.11***	37522.6***	5273.7***	12153.9***
GxE	54	73.42***	77.24***	83.51***	2352.1***	914.3***	1283.3***
Error	57	0.001	0.001	0.001	276.6	143.3	424.7

The results of this study indicated that great variations could be ascribed to different environmental conditions, environment being the dominant factor contributing to total variations of AX fraction content. These results are generally consistent with the results of Lempereur *et al*. (1997) who showed the influence both of cultivar and environment for TOAX and WEAX fractions in durum wheat grains. For ARs, STPC and AA the environment (E) was the main factor contributing to the total variation of the considered parameters. These results are consistent with Menga *et al.* (2010), who observed a strong impact of environment on STPC in durum wheat; the results are also in agreement with a study of Mpofu *et al.*, 2006 on spring wheat. Moore and Yu, (2008) observed effects of both E and G on the STPC and AA in wheat bran, higher than the G × E interaction. Yu *et al.* (2004) in soft wheat flour samples reported that the DPPH radical scavenging activity and phenolic content were significantly influenced by the variety (G) and growing location (E) and Andersson *et al*. (2010) also reported a strong influence of genotype as well as environment on the content and composition of ARs in bread wheat.

5.1.4.1 Environment variability for the examined bioactive compounds.

Box plots were used to graphically visualize the variations of the measured parameters within and between environments (Figure 10). On average, the highest

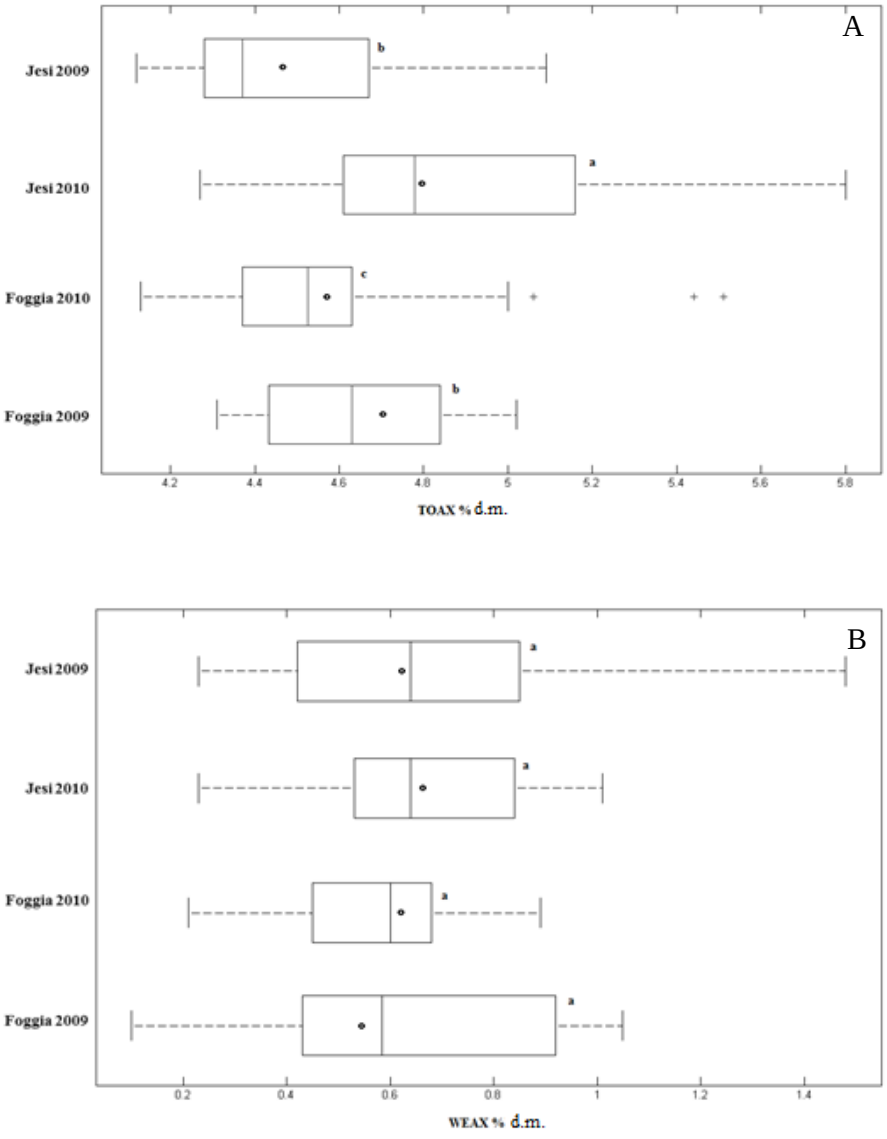
TOAX values were found in Jesi10 (4.9 % d.m.) with a range between 4.3 and 5.8% d.m. and in Foggia09 (4.6 % d.m.) with a range between 4.3 and 5.1% d.m.; in these two experiments the highest TOAX contents are also associated to the highest level of rain (Figure 10 A). In general, the lowest values of WEAX were evidenced in the material grown in Foggia09 (0.5 ± 0.5% d.m.), however, on average, among the four environments the range in WEAX was very limited (Figure 10 B).

These results were in agreement with the observations of Li *et al.*, (2009), who found the highest level of TOAX in the environments where higher amounts of rainfall occurred. The influence of water availability on AX accumulation was discussed by Dornez *et al.* (2008), who in common wheat grain evidenced significantly higher amount of WEAX during the rainy years. Other studies on common wheat have also reported environmental effects on the level of AX fractions (Gebruers *et al.*, 2010; Dornez *et al.*, 2008; Anderson *et al.*, 1993; Coles *et al.*, 1997). Gebruers *et al.* (2010) observed different variations related to environment climatic conditions concluding that the total variation in the AX levels was determined by the interaction with other factors such as agronomical input and soil type.

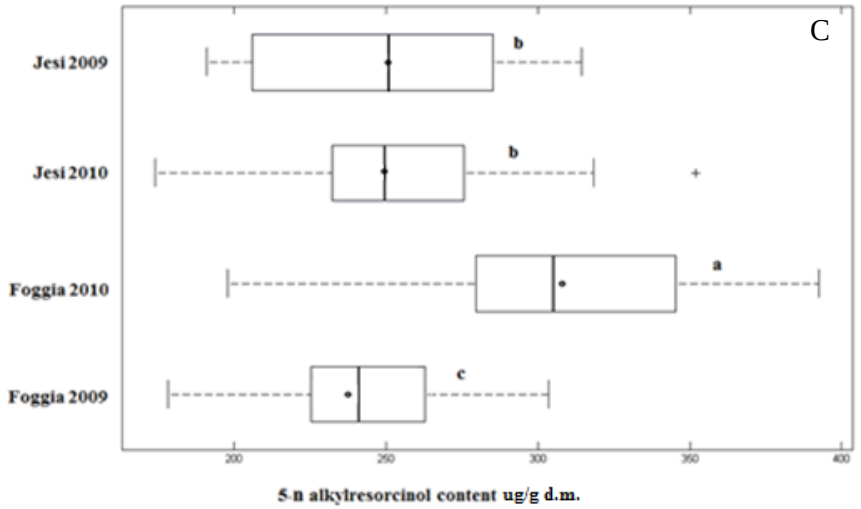
Between the environments, the AR content (Figure 10C) showed a significantly higher mean value in Foggia10 (310 μg/g d.m.) with a range between 194 and 393 μg/g d.m. In particular, the lowest AR content (241 μg/g d.m.) was recorded in Foggia09 and the highest in Foggia10, which is the location where the lowest amount of precipitation occurred. Andersson *et al.* (2010) reported great variability in AR content of bread wheat between year and locations, with highest contents in an environment characterized by hot dry conditions during grain filling.
As regard STPC, the lowest mean value was recorded in Foggia10 (99 μg/g d.m.) and the highest ones in Foggia09 (130 μg/g d.m.) and in Jesi10 (132 μg/g d.m.) (Figure 10D). It should be noted that in the present study high STPC and low ARs were recorded for the environments characterized by high rainfall level during the growing season. The means for AA (Figure 10E) significantly varied between environments, from 109 (Foggia09) to 150 (Jesi10) EC50 (mg of wheat milled grain on d.m. basis required to obtain 50% DPPH scavenging).

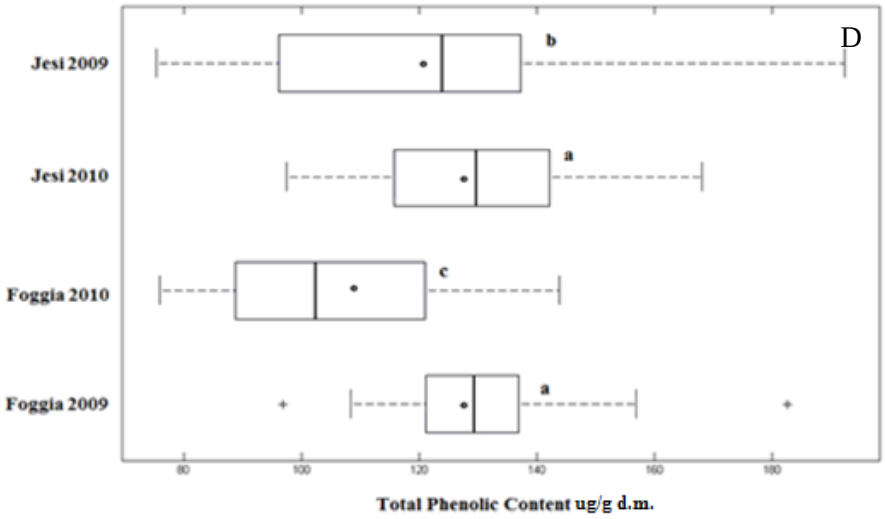
Roberto Ciccoritti

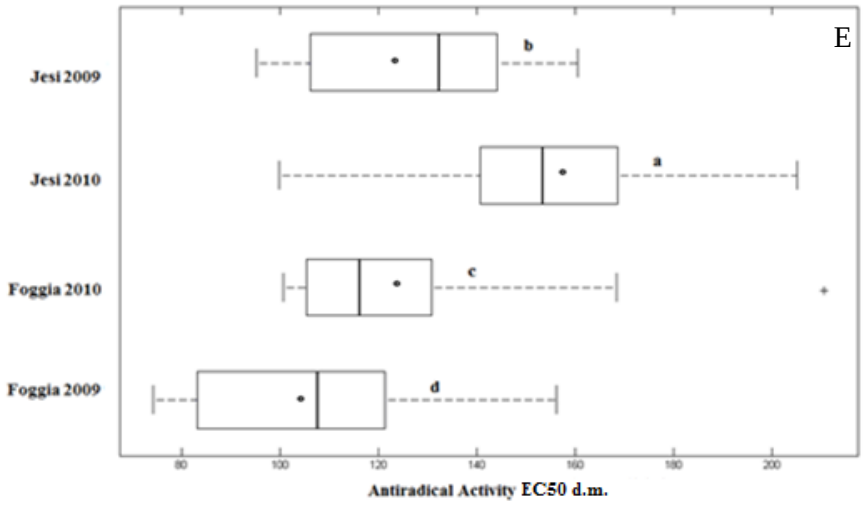
Figure. 10 Dataset box plots: evaluation of environmental variability. A. TOAX (Total Arabinoxylans); B WEAX (Water Extractable Arabinoxylans); C AR (Alkylresorcinol content); D. STPC (Total soluble phenolic content); E. AA (Antiradical activity). Different letters indicate that averages are significantly different from each other ($p < 0.05$). Box plot explanation: right edge of the box, 75th centile; left edge, 25th centile; vertical bar within box, median; right vertical bar outside box, maximum value; left vertical outside box, minimum value Points inside the box are average value. Points outside the box are outliers or suspected outliers.



Roberto Ciccoritti







Roberto Ciccoritti

Yu *et al.*, (2004), did not detect any relationships with specific environmental factors, including total solar radiation, daily average solar radiation or the hours exceeding 32°C. In the present study found a significant influence of E on the AA, but no relationship between AA and total rainfall.

5.1.4.2 Genetic variability for the examined bioactive compounds.

The difference of TOAX among 19 varieties based on Duncan's test are reported in Figure 11. Significant variations existed among the durum wheat varieties for TOAX (Figure 11A) in particular mean values of genotypes across environments ranged from 4.3 to 5.2% d.m. The TOAX range was comparable to the results obtained by Lempereur *et al.* (1997) with a different methods of analysis.

The results showed that, with regard to TOAX and WUAX (Figure 11B) content some cultivars were significantly superior to the others: Trionfo, followed by Alemanno, presented the highest means values for TOAX, having consistently the highest TOAX content in three of the four environments analyzed (*data not shown*). Tirex and Saragolla had lowest level of TOAX and WUAX. Trionfo, Tirex, Simeto, Ciccio, Arnacoris and Imhotep presented higher variability as regard the TOAX.

The genotypes differed considerably in their AR contents (Figure 11C) but not significant difference was observed in the homologue composition (*data not reported*). On average, the values ranged from 189 (Normanno) to 330 μg/g d.m. (Iride). The box plots show a limited variation in some genotypes (Creso, Iride, Minosse, Normanno, Simeto, Tirex), underlying the strong contribution of genotype to the AR content.

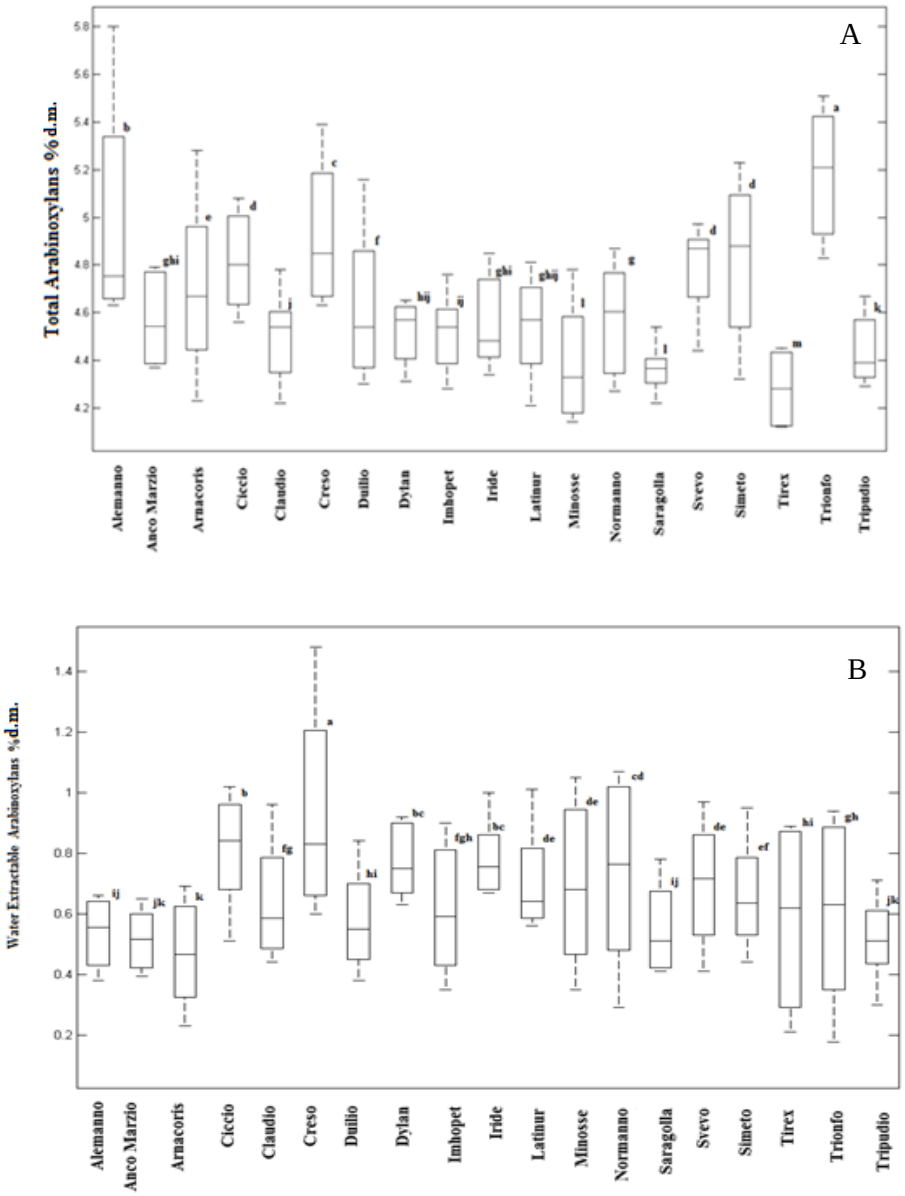
The STPC values (Figure 11D) ranged from 99 (Minosse) to 145 μg/g d.m. (Trionfo) a broad range of variation was also observed for Anco Marzio, Imhotep and Simeto.

The AA (EC_{50} = mg of wheat milled grain, on d.m. basis, required to obtain 50% DPPH scavenging.) mean values calculated across replicates and environments ranged from 100 (Tirex) to 157 (Alemanno).

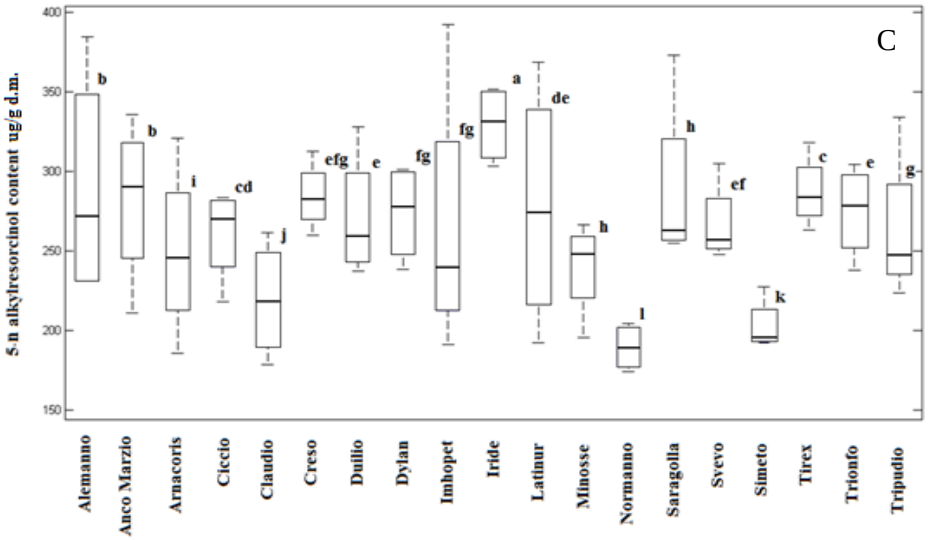
Most of the genotypes, except Alemanno, showed a broad range of variation in AA content due to environment (Figure 11E).

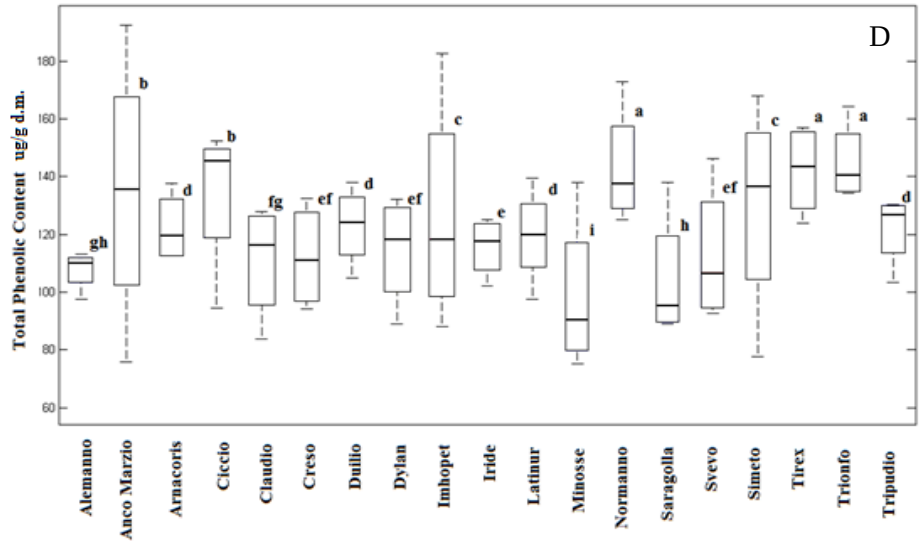
Roberto Ciccoritti

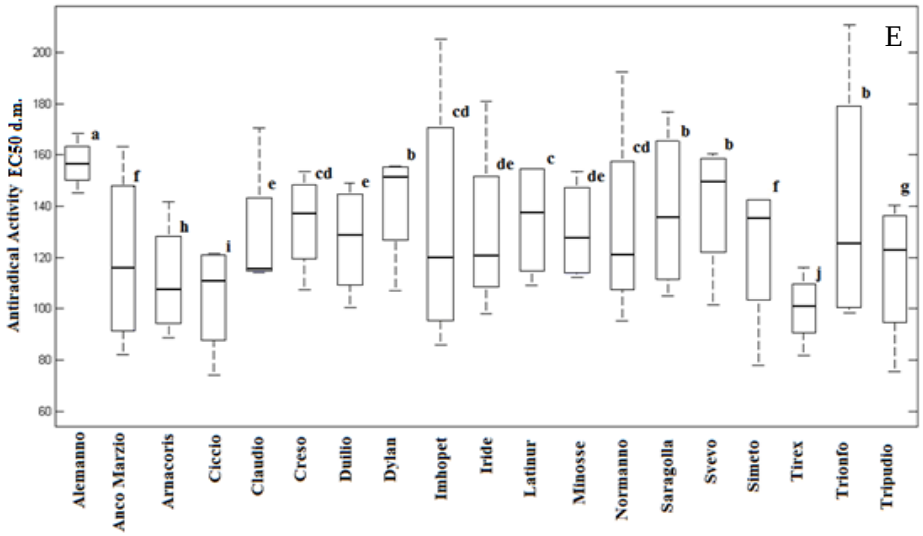
Figure. 11. Dataset box plots: evaluation of variety variabilityA) Total Arabinoxylans, B) Water extractable Arabinoxylans; C) 5-n-alkilresorcinol; D) Total phenolic content; E) Antiradical Activity. Different letters indicate that averages are significantly different from each other ($p < 0.05$). Box plot explanation: right edge of the box, 75th centile; left edge, 25th centile; vertical bar within box, median; right vertical bar outside box, maximum value; left vertical outside box, minimum value. Points outside the box are outliers or suspected outliers.



Roberto Ciccoritti





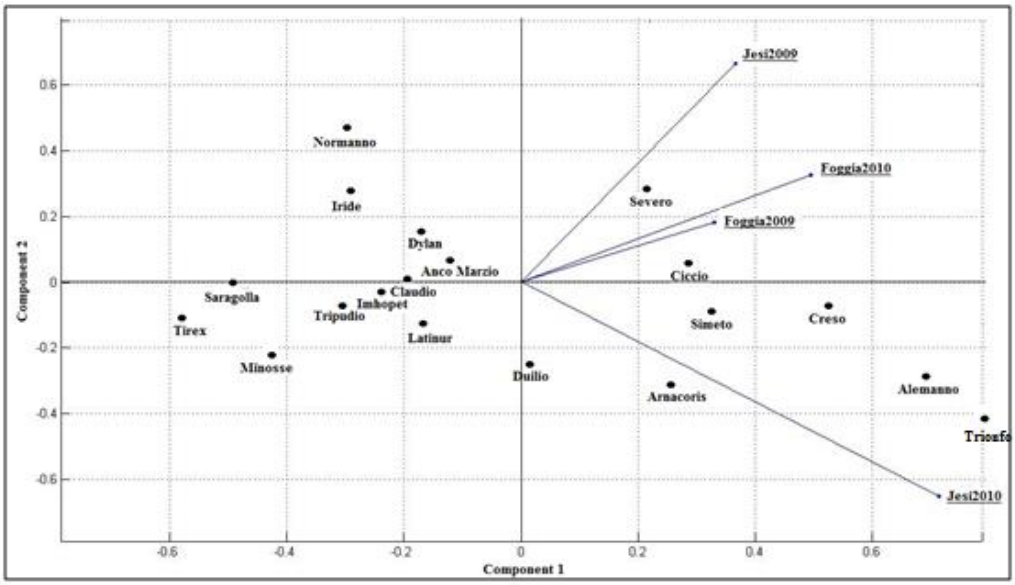


Roberto Ciccoritti

5.1.5 Genotype and environment interaction in TOAX and AR accumulation (Principal Component Analysis, PCA)

As previously discussed, genotype and environment strongly affected AX and AR concentrations of durum wheat whole grain, the variability due to G × E interaction was lower than that of genotype or environment. The results, however, showed that the varieties did not respond similarly to different environmental conditions and, in agreement with the results of Gebruers *et al.*, (2010) in soft wheat, some varieties tended to have consistently high or low AX contents. In order to visualize the relationships between the genotypic performance in the different environments, Principal Component Analysis (PCA) was used and bi-plot of first two components was reported (Figures 12).

Figure 12 Genotype × environment bi-plot from principal component analysis for TOAX (total arabinoxylans) of 19 durum wheat varieties grown in four environments.



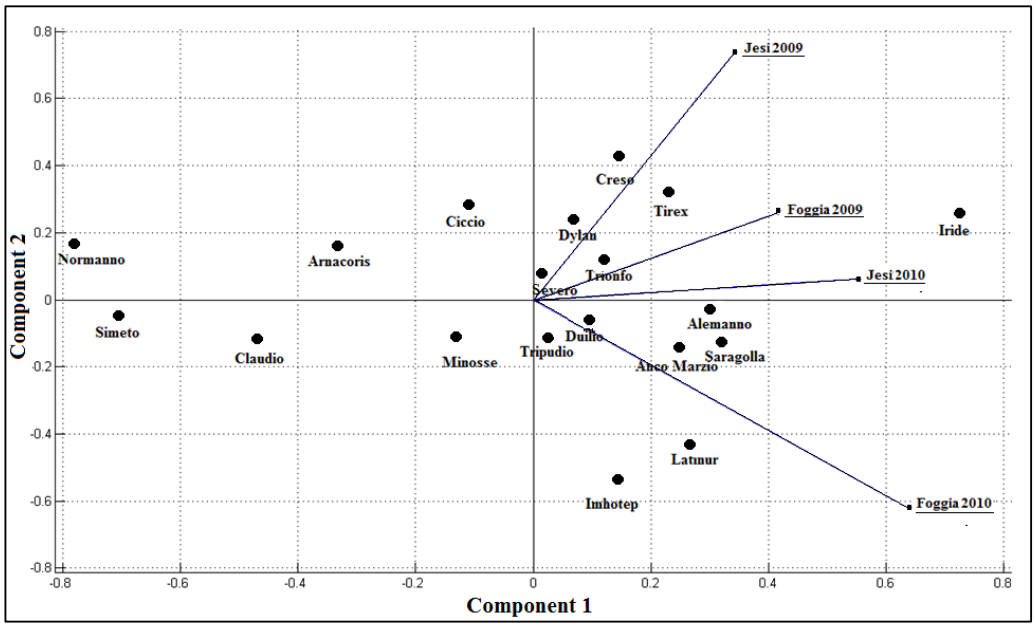
As regard TOAX the first two principal components explained about 83% of total variation. The PCA applied on varieties and environments for TOAX fractions were plotted on bi-plots. All the environments fell onto the positive side of PC1, and Jesi2010 alone was located on the negative side for PC2. The eleven varieties which were relatively stable across environments fell close to the PC2 axis; while Saragolla

Roberto Ciccoritti

and Tirex were below average in all environments, ranging between 4.5 to 4.1 d.m. Varieties which fell on the right side of PC1 were characterized by very strong interaction with the environment, presenting their maximum potentiality of TOAX accumulation in Jesi2010; in fact, for these varieties the range of the observed values was from 5.1 (Duilio) to 5.8% d.m. (Trionfo).

As regard AR the effect of the environment can be clearly seen by PCA applied to varieties and environments (Figure 13).

Figure 13. Genotype × Environment bi-plot from PCA for AR (5-n-alkylresorcinol) content of 19 durum wheat varieties grown in four environments.



The environments are in similar positions on the positive side of PC1 (explaining 66% variance) and only Foggia 2010 is located on the negative side for PC2 (explaining 30% variance). The varieties with positive scores for PC1 and PC2 generally had AR contents slightly above the average. Among them, Iride alone (positive value for PC1) showed higher and more stable contents of ARs across the environments (range: 304–352 µg/g d.m.). In addition, Normanno (AR range: 174–204 µg/g d.m.) and Simeto (AR range: 192–227 µg/g d.m.) appeared to be less affected by the environment but had low ARs content.

Roberto Ciccoritti

Seven varieties with positive scores for PC1 showed very strong interactions with the environment and had the highest contents of ARs in Foggia 2010; in this environment, the varieties had a low variation range min 328 (Duilio) and maximum 393 μg/g d.m. (Imhotep).

Table 5 summarizes the whole grain average compositions of a set of 10 durum wheat varieties selected on the basis of their high mean values for more parameters. Ciccio, Severo and Trionfo presented a good grain composition and high concentrations of most of the examined parameters. Moreover, Iride, previously reported for the good AR stability in the different environments, showed the highest AR content and good levels of WEAX and AA

Table 5. Whole grain composition of some of the examined durum wheat varieties (mean values on d.m. and St.Dev.).

Samples	**TOAX %**	**WUAX %**	**WEAX %**	**ARs μg/g**	**STPC μg/g**	**AA ****
Alemanno	4.99±0.49	4.46±0.46	0.54±0.11	294 ±70	107±7	156±13
Anco Marzio	4.57±0.20	4.06±0.11	0.51±0.10	290±31	134±45	119±37
Arnacoris	4.71±0.38	4.24±0.51	0.47±0.17	227±23	122 ±10	111±22
Ciccio	4.82±0.21	4.00±0.33	0.81±0.18	279±30	134±27	104±21
Creso	4.93±0.31	3.97±0.08	0.93±0.35	280±32	112±17	133±23
Iride	4.56±0.20	3.78±0.13	0.78±0.12	324±36	115±10	130±36
Severo	4.79±0.19	4.09±0.07	0.70±0.21	268±35	113±23	140±29
Simeto	4.82±0.35	4.16±0.44	0.66±0.18	199±19	129±35	122±28
Tirex	4.28±0.16	3.70±0.34	0.58±0.31	283±36	142±17	100±20
Trionfo	5.18±0.27	4.58±0.34	0.61±0.30	271±39	144±13	139±64
Average* ±St.Dev.	4.64±0.23	3.99±0.24	0.65±0.12	259±34	121±13	127±14

* average of all the 19 durum wheat varieties

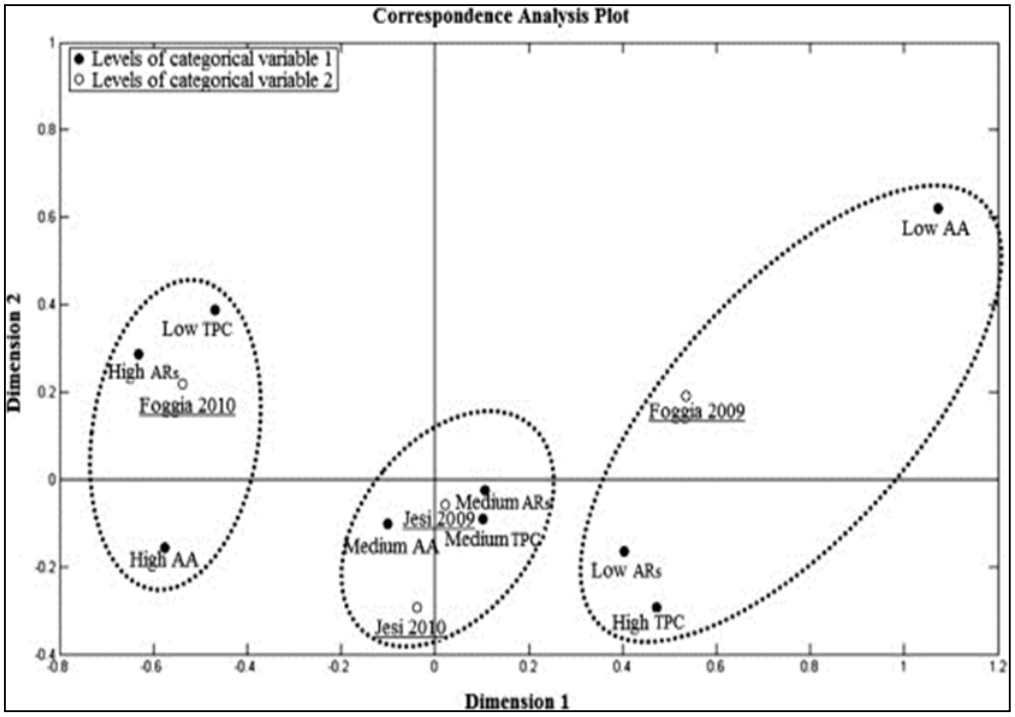
**EC50 = mg of wheat milled grain (on DM basis) required to obtain 50% DPPH scavenging.

5.1.6 Environmental profile in relation to the accumulation of the ARs, STPC and to AA in durum wheat whole grain (Correspondence Analysis)

Correspondence analysis was applied to summarize in a bi-dimensional plot the influence of different environments on the phytochemical profile of durum wheat

grain. The whole data sets (30 durum wheat varieties × 4 environments) for ARs, STPC and AA were converted to a frequency table with the environment as the first variable and three arbitrary categories for each trait: low, medium, high second variable. Graphical representations of the frequency table and the limits of the arbitrary category, based on means values and standard deviations, are represented in figure 14.

Figure 14. Scatter plot of the correspondence analysis performed between: A) ARs (5-n-alkylresorcinols), STPC (Total soluble phenolic content), AA (antiradical activity) arbitrary category and environment variables. The range of the arbitrary categories are the follow: ARs, low≤200.00, medium≥200.10≤300.00, high≥300.10; STPC, low≤100.00, medium≥100.10≤150.00, high≥150.10; AA, low≤90.00, medium≥90.10≤180.00, high≥180.10.



The distances between the points that represent the environments are a measure of the similarity among the environment-frequency profiles. Each trait-category point will lie close to the environment for which the trait-category is prominent. For AR, STPC and AA the correspondence analysis revealed three main groups (Figure 11).

The closeness of the AR, STPC and AA medium categories to Jesi 2009 and Jesi 2010, indicates that these categories are "strongly" associated with these

Roberto Ciccoritti

environments and that the medium category is prominent for all parameters. The closeness of High ARs, Low STPC and High AA to Foggia 2010 indicates the higher frequency of these categories in this environment, while High ARs and Low STPC determine the position of Foggia 2010 on the plot. The fact that Foggia 2010 is located relatively far from the other environments indicates that the climatic conditions recorded during grain filling and harvesting in this environment strongly affected the phytochemical profile of the grain. Differently Foggia 2009 is graphically located near Low AA and close to Low ARs and High STPC, that could be explained by not usual climatic condition in this environment such as an exceptionally high amount of total rainfall.

5.2 *Variations (amount and chemical composition) in phytochemicals and in the total AA of grain among different Triticum species.*

5.2.1 Samples and experimental design.

In order to evaluate the variability of the examined phytochemicals in different *Triticum* species two lines and two cultivars of *T. turgidum* ssp *dicoccum*, two lines and one cultivar of *T. monococcum*, four cultivars of durum (*T. turgidum* ssp *durum*) wheat and four cultivars of common wheat (*T. aestivum*) were considered (Table 6).

All crop species were grown during 2011-2012 at the experimental field of CRA-QCE, (Montelibretti RM).

The experimental design was a randomized block with three replications. The elementary plot of 10 m^2 consisted of eight rows, 17 cm apart, sown with 400 germinating kernels/m^2. The husbandry conditions were applied according to local practice, including 150 kg/ha of nitrogen (N) applied in three top dressings.

In addition, due to the limited availability, Khorasan wheat, *T. turgidum* ssp *turanicum* (*Kamut* ®) and single accessions of both *T. timopheevii* and *T. zhukovskyi* were grown, in same years an environments, in two replicates of a single row with 40 plants, 8 cm apart within the row and 25 cm between rows. This last group of wheats was not submitted to multivariate analysis and discussed separately. Field replicates were collected for the chemical analysis.

Table 6. Wheat genotypes analysed and their genome composition.

Common name	Species and subspecies	Genoma	Accession or cultivar
Soft wheat	*Tritricum aestivum ssp vulgare*	A^UBD	Aubusson-Bilancia Blasco-Sagittario
Durum wheat	*Tritricum turgidum ssp durum*	A^UB	Iride-Normanno Simeto-Tirex
Emmer	*Tritricum turgidum ssp dicoccum*	A^UB	Prometeo-Ersa Filosini 5563
Einkorn	*Tritricum monococcum ssp monococcum*	A^M	Stendhal ID331 Monlis
Khorasan wheat	*Tritricum turgidum ssp turanicum*	A^UB	Kamut
Thimopheevi wheat	*Tritricum thimopheevi*	A^UG	Far 72
Zhukovsky wheat	*Tritricum zhukovsky*	A^MA^UG	Far 75

5.2.2 Phytochemical profile and AA of different *Triticum* species

Milled grain from genotypes belonging to 7 *Triticum* species were compared for their AR, STP and AA contents. Results from ANOVA of the soft, durum, *dicoccum* and *monococcum* wheats showed a statistically significant effect of the wheat species on the phytochemical profile ($p < 0.001$) (Table 7).

Moreover, univariate ANOVA revealed that the species had a statistically significant effect on both total AR and STP content ($p < 0.001$). By contrast, there were not significant differences in the AA among the wheat species analyzed. In particular a wide variation in AR content were presented, especially between *T. turgidum* ssp *durum* genotypes, which, on average, had the lowest mean AR content (286 µg/g d.m.), and *T. turgidum* ssp *dicoccum*, which had the highest mean AR content (377 µg/g d.m.). The present results are slightly lower than those reported by Andersson *et al.*, (2008) in different wheat species harvested in Hungary in 2005, probably because of genotype, environment and genotype × environment effects. However,

the differences in AR content between the wheat species grown in Hungary are similar to those observed in the present study. The STP contents in the genotypes of *T. aestivum*, *T. turgidum* ssp durum, *T. turgidum* ssp dicoccum and *T. monococcum* varied in the ranges 93–207, 115–148, 47–97 and 60–97 μg of catechin equivalents/g, respectively.

Table 7. Phytochemical composition and antiradical activity (mean ± SE) of four wheat species.

Trait	*T. aestivum ssp vulgare*	*T. turgidum ssp durum*	*T. turgidum ssp dicoccum*	*T. monococcum ssp monococcum*
ARs [a]	321±18[b]	286±11[b]	377±17[a]	334±8[a,b]
STP [b]	114±12[a]	141±11[a]	74±7[b]	79±6[b]
AA [c]	87±3[a]	90±3[a]	88±11[a]	93±8[a]
C17:0[d]	5.7±1.0	2.5±0.3	1.0±0.2	1.1±0.6
C19:0 [d]	31.7±3.0	12.3±2.2	12.1±2.7	13.4±2.7
C21:0 [d]	41.9±1.7	50.4±2.6	48.1±2.5	44.1±3.5
C23:0 [d]	17.8±3.4	27.3±2.8	27.8±1.9	30.0±1.4
C25:0 [d]	4.1±0.8	7.5±1.9	11.0±3.0	11.5±1.7
C17:0/C21:0	0.14±0.03	0.05±0.01	0.02±0.00	0.03±0.01

[a] ARs: 5-n-alkylresorcinols, results are expressed as μg ARs/g wheat milled grain (d.m. basis).

[b] STP: Soluble Total Polyphenols, results are expressed as μg cathechin equivalents /g of wheat milled grain (d.m. basis).

[c] AA Antiradical Activity, results are expressed in terms of EC_{50} = mg of wheat milled grain (d.m. basis) required to obtain 50% DPPH scavenging.

[d] Expressed as %.

On average, the ancient wheat crops *T. monococcum* and *T. turgidum* ssp dicoccum did not significantly differ from each other in STP content; same situation was observed for the modern durum and common wheat lines. On average, the STP content in the ancient wheat crops was about half of that measured in durum or common wheat.

As regard the AA, in average, not significant differences, were found for DPPH, and AA values ranging from 87 to 93 EC50 (EC50 = mg of wheat milled grain on d.m. basis required to obtain 50% DPPH scavenging.), Table 7. The lowest and highest AA values of 44 and 122 EC50, respectively, were determined for *dicoccum* samples, the range of variation among these lines being 78 EC50. Previous studies

showed that ARs do not exert high antioxidant activity in the DPPH radical assay (Stasiuk and Kozubek, 2010). Interestingly, the lines with the highest (line 5563) and the lowest (line Ersa 6) free radical scavenging activity were *dicoccum*-breeding lines at an advanced stage of selection. This is in agreement with previous findings of Abdel-Aal and Rabalski, (2008) that observed broad range of AA in wheat largely due to genotypic variations. *T. durum, T. dicoccum* and *T. monococcum* genotypes revealed similar values of AR homologues; if compared with common wheat, these species showed a great proportion of higher homologues C21:0, C23:0 and C25:0. By contrast, the acetone extracts of *T. aestivum* contained a higher proportion of C17:0 and C19:0 homologues, which represented 5.7% and 31.7% of the AR pool, respectively.

The differences in AR compositions between durum and common wheat observed in the present study were similar to those reported by other authors (Knodler *et al*., 2010; Landberg *et al.,* 2009). In the four *Triticum* species analysed (*T. durum, T.Aestivum, T. dicoccum* and *T. monococcum),* C21:0 and C17:0 were the more and the less frequent AR homologues, respectively, whereas the C17:0 homologue showed the lowest range of variation. In a previous report (Ross *et al*., 2003), the C17:0/C21:0 ratio was used to distinguish between different cereal species. In this study, the ratio between C17:0 and C21:0 ranged between 0.01 (*T. monococcum*, line ID331) and 0.18 (*T. aestivum*, cv. Blasco); *Triticum dicoccum* showed the lowest average ratio of 0.02, and the lowest variation (0.02–0.03) for this parameter. The highest average ratio (0.14) combined with the greatest variation was found in *T. aestivum* (0.10 in cv. Sagittario to 0.18 in cv. Blasco).

5.2.3 Modern wheats *vs* ancient *T.* species) in relation to phytochemical profile and AA (Principal Component Analysis, PCA)

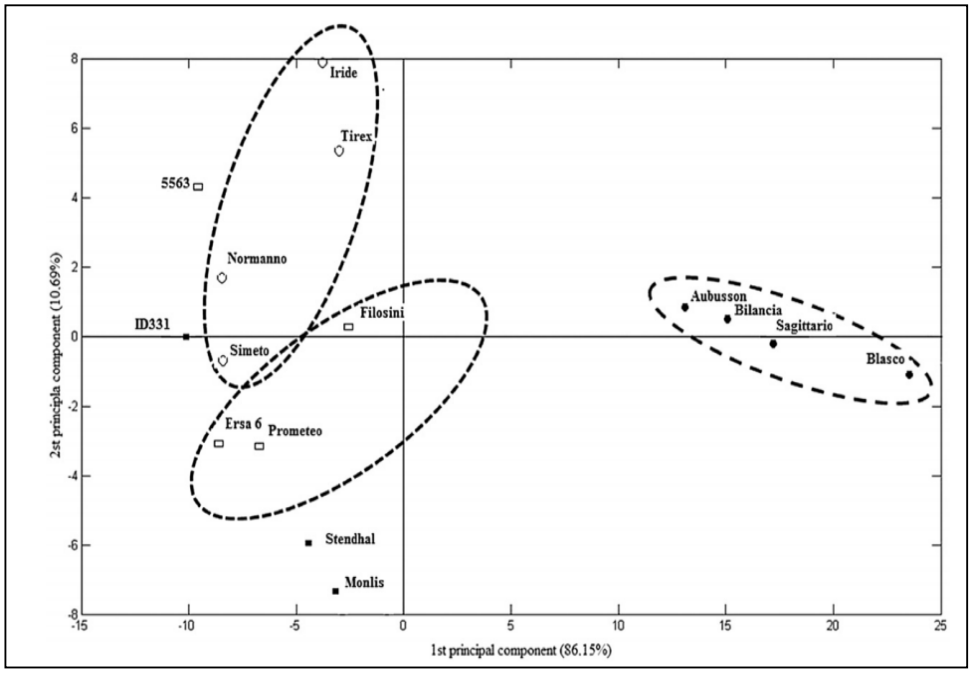
Modern durum and common wheat varieties were also compared with the ancient *monococcum* and *dicoccum* wheats using PCA. Two proper matrix was constructed: the first one on the basis of the AR homologue patterns and the C17:0/C21:0 ratios observed in the same extracts (Figure 15), and the second on the basis of the original data set reflecting AR, STP content and AA in the acetone extracts (Figure 16). The score plot (Figure 15), obtained by combining the information from AR compositions, showed that the two principal components (PC1

and PC2) accounted for 86.2% and 10.7% of the total observed variance, respectively.

The samples occurred in two major clusters, each showing two subpopulations in agreement with their taxonomic origins.

All *monococcum* and *dicoccum* genotypes had positive scores for PC1, being the AR content the main variable explained by the first component (factor loading: 0.90), except for two genotypes, *dicoccum* cv. Prometeo and *monococcum* line ID331

Figure. 15. Score plot of the two principal components (PC1 and PC2) showing the distribution of four *Triticum* species for their AR homologue chains (C17:0, C19:0, C21:0, C23:0, C25:0) and C17:0/C21:0 ratios. ● *T. aestivum;* ○ *T. turgidum ssp durum;* □ *T. turgidum ssp dicoccum;* ■ *T. monococcum*.



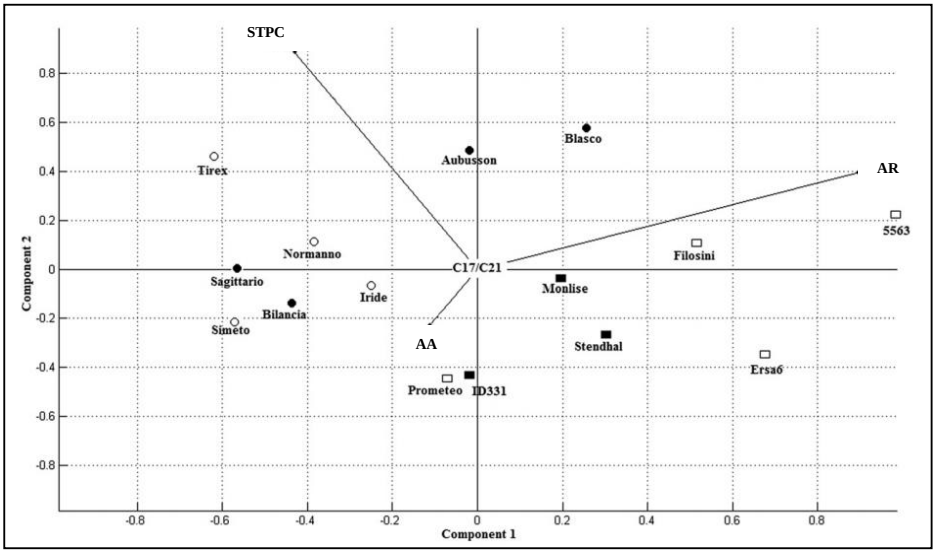
On the other hand, the observed variation in AR composition (Figure15) was found to be correlated with the taxonomic origin of each sample according to the effect of the C19:0 homologue (factor loading: 0.80) along PC1 and, to a minor extent, according to the effect of the C21:0 homologue (factor loading: 0.79) along PC2. The C19:0 homologue and the C21:0 homologue were shown to explain 86.1% and 10.7% of the variance, respectively. The score plot of Figure 15 *T. aestivum* was

Roberto Ciccoritti

located in the right side of PC1, while the other three wheat species were located in the left quadrants.

The scores for the first two components determine the position of each sample in the PCA bi-plot (Figure 16), while the value of each analyzed parameter is determined by the vector from the bi-plot origin to the sample position. The first component (PC1), which accounted for 67.2% of total variance, was strongly associated with ARs, while the second component (PC2) was mainly associated with STP content.

Figure. 16. Bi-plot from principal component analysis (PCA) of 15 genotypes from four *Triticum* species, on total phenol (STP) content, 5-n-alkylresorcinol (AR) content, antiradical activity (AA, expressed as EC50) and C17:0/C21:0 ratio. ● T. *aestivum;* ○ *T. turgidum ssp durum;* □ *T. turgidum ssp dicoccum;* ■ *T. monococcum*



The bi-plot showed a negative correlation between AR and STP content and a negligible effect of the C17:0/C21:0 ratio on the PCA discriminating power of PCA. Interestingly, the C17:0/C21:0 ratio, proposed as a molecular tool to distinguish between different wheat species and rye (Andersson *et al.,* 2008), allowed low discriminating power between the wheat species analyzed here.

Roberto Ciccoritti

5.2.4 Phytochemical profile and AA of Khorasan, Timopheevi and Zhukovskyi wheats

Likely for other ancient wheat crops, there is growing interest for khorasan wheat (*T. turgidum* spp turanicum), due to its claimed nutritional quality and versatility. This AB-genome species is a spring wheat early in maturity, mainly cultivated in Afghanistan, Iran, Turkey and Turkmenistan, in the Turan plain. To the best of our knowledge, there are no data on the polyphenol profile, AR content and AA of this species.

Timopheevi wheat (*T. timopheevii*) is a tetraploid AG-genome spring wheat mainly distributed in Transcaucasia. Its late-maturing kernels are medium long, slender and hard. Timopheevi wheat is the result of a recent interbreeding between A^u-genome *Triticum uratu* and G-genome Aegilops speltoides. Finally, a natural cross between *T. timopheevii* and A^m-genome *T. monococcum* would have resulted in the novel amphiploid A^uA^mG-genome *Triticum zhukovskyi*, as suggested by its morphological traits, geographic distribution, karyotype and meiotic behaviour (Bowden, 1959).

The phytochemical profile, AA, AR homologue composition and C17:0/C21:0 ratio of these species are reported in Table 8. The AR contents of Timopheevi and Khorasan wheats were similar and slightly higher than that of Zhukovskyi wheat. This latter species also showed the highest STP content followed by the Timopheevi and Khorasan wheats. Interestingly, the STP contents of these three species were approximately two-fold higher than those of durum and common wheats, and about three times higher than those of the ancient *monococcum* and *dicoccum* wheat crops (Table 7). These large differences in STP content could be accounted for by the different growing conditions of Khorasan, Timopheevi and Zhukovskyi wheats as compared with the *monococcum* and *dicoccum* wheats.

No significant correlation was found between STP content and AA. In particular, Khorasan wheat showed the highest AA, which, however, was low if compared with those of other wheat species grown in the same environment (Table 8).

Therefore, Khorasan, Timopheevi and Zhukovskyì wheats were found to possess poor scavenger properties compared with durum, common, *dicoccum* or *monococcum* wheats.

Table 8. Phytochemical composition and antiradical activity (mean ± SE) of timopheevi, zhukovsky and khorasan wheats.

Trait	*Khorasan wheat*	*Timopheeve wheat*	*Zhukovsky wheat*
ARs [a]	327±25	332±9	306±13
STP [b]	215±10	250±10	286±11
AA [c]	103±7	132±4	131±8
C17:0[d]	4.4±0.3	2.5±0.1	6.4±1.4
C19:0 [d]	24.4±0.8	20.6±0.1	63.7±0.3
C21:0 [d]	40.3±0.2	42.4±0.4	25.8±2.4
C23:0 [d]	24.1±0.4	26.7±0.3	4.0±0.6
C25:0 [d]	6.7±0.2	7.7±0.2	n.d.
C17:0/C21:0	0.11±0.0	0.06±0.0	0.25±0.1

[a] ARs: 5-n-alkylresorcinols, results are expressed as µg ARs/g wheat milled grain (on d.m. basis).

[b] STP: Soluble Total Polyphenols, results are expressed as mg cathechin equivalents /Kg of wheat milled grain (on d.m. basis).

[c] AA Antiradical Activity, results are expressed in terms of EC_{50} = mg of wheat milled grain (on d.m. basis) required to obtain 50% DPPH scavenging.

[d] Expressed as %.

Interestingly, *T. zhukovskyi* showed an unusual AR homologue composition; in particular, this species was found to lack the C25:0 homologue and have the highest proportion (about 64%) of the C19:0 homologue, approximately 3-fold higher than that of the other *Triticum* species. This result is very interesting because previous studies demonstrated that long-chain resorcinolic lipids affect its activity on protein structure (Kozubek *et al.*, 1992; Stasiuk *et al.*, 2008). This effect was probably modulated by the structure of the resorcinolic molecule, in particular by the length of its alkyl chain.

On the basis of the results, the hierarchy of the C17:0/C21:0 ratio was Zhukovsky wheat >> Khorasan wheat >> Thimopheevi wheat. Khorasan wheat showed a C17:0/C21:0 ratio (0.11) similar to that of common wheat (0.14). Finally, because of the wide variation in the phytochemicals and in AR homologue pattern, these species could be interesting for production of cereal foods for prevention of cardiovascular diseases and cancer.

5.3 Effects of technological treatments on potential nutritional quality of the durum wheat end-products.

5.3.1 Samples

For this study five different Italian durum wheat genotypes (Claudio, Meridiano, Saragolla, Strongfield and Svevo) kindly provided by Produttori Sementi Bologna (PSB Bologna, Italy) were used. Part of the grain samples (20 g) were milled to pass 0.5 mm screen using a laboratory cyclone mill (Cyclotec 1093, FossItalia) and used to characterize the kernels; a different portion (10 kg) was used to technological treatments and then analyzed.

In table 9 the TOAX, WEAX and AR content of the analyzed samples was reported. High variability among the five examined cultivars was observed: as regard TOAX and WEAX, Meridiano showed the highest value (5.95 and 0.70% d.m., respectively), while for the AR Saragolla presented the highest value (301 μg/g d.m.).

Table 9. Whole grain composition (TOAX, WEAX and AR) of the five durum wheat varieties (mean values on d.m. and St.Dev.).

Varieties	TOAX %	WEAX %	ARs μg/g
Claudio	4.02±0.05	0.42±0.09	101±7
Meridiano	5.95±0.09	0.70±0.08	142±11
Saragolla	4.79±0.10	0.42±0.10	301±7
Strongfield	3.67±0.05	0.31±0.08	124±11
Svevo	4.50±0.09	0.68±0.05	109±6

5.3.2 Influence of hydrothermal, micronization and air classification processes on AR, TOAX and WEAX contents of whole grain.

A portion of the five cultivars, was used for hydrothermal process and subjected to following technological processes to evaluate the influence of practice on bioactive compounds under study. Micronization of durum wheat grains (AS IS

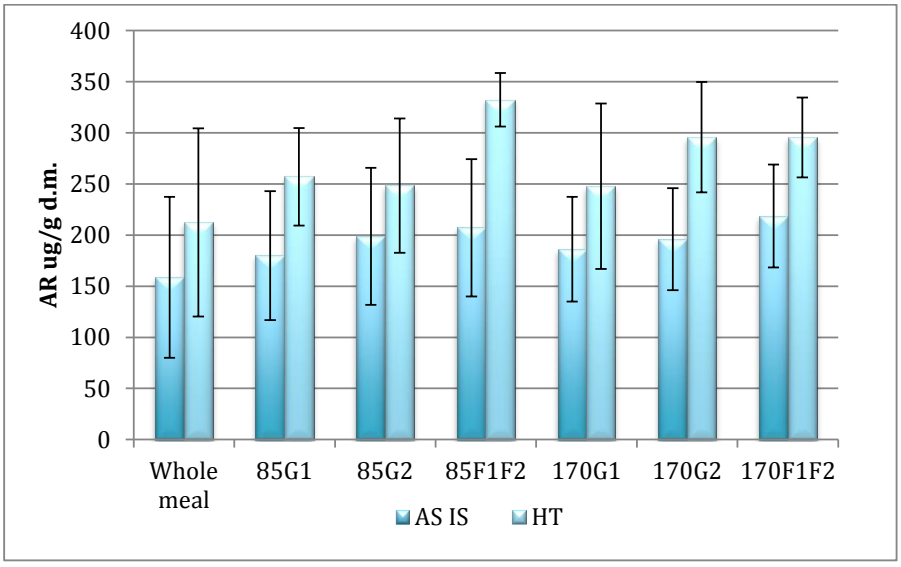
and hydrothermally processed) carried out at two peripheral speeds (85 and 170 Hz) provided two fine flours with different particle size. For each sample as result of the air classification process, three flour fractions for each peripheral speed were obtained (G1, F1 and F2). Moreover, the air classification process was replicated on F1 fraction by obtaining another fraction (indicated as G2); then, the residual F1 fraction was mixed with F2 fraction (indicated as F1+F2 fraction).

The total AR content (μg/g) of the all samples obtained from five durum wheat varieties are presented in Figure 17 as mean values. Total ARs showed a range of variation between 159 (wholemeal) and 219 μg/g (170 F1+F2 fraction) for material "AS IS" and between 213 (wholemeal) and 332 μg/g (85 F1+F2 fraction) for samples hydrothermally processed (HT).

The highest amount of AR in G2 fraction confirmed the location of these compounds in the kernel. In fact, previous studies (Landberg *et al*., 2007; Borron *et al*., 2011) reported the preferential presence of phenolic compounds in intermediate layer and a high content of AR in pure intermediate layer.

The hydrothermal treatment determined significant increase of AR content in all materials. Similar results have been reported by Cheng *et al*. (2006) and by Gunenc *et al.* (2013) as an effect of heat stress on conjugated polyphenolic compounds.

Figure 17 Influence of technological processes on AR (5-n alkylresorcinol) contents (mean values of five durum wheat varieties). Bars represent standard deviations.



Roberto Ciccoritti

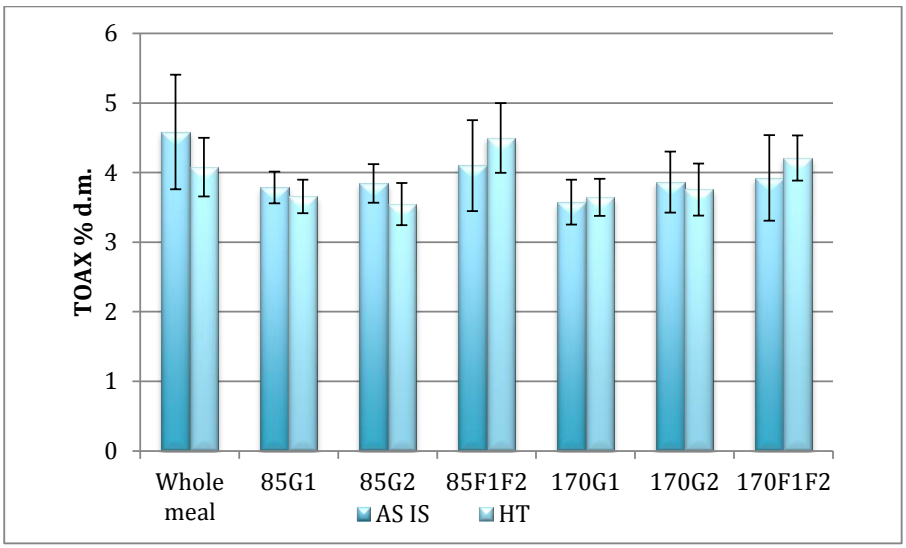
The data of this study suggested that the higher amount of ARs in treated kernels (hydrothermal process) in comparison with "AS IS" ones could be attributed to their increased extractability due to heat treatment. It's interesting to note that technological processes did not influence significantly the AR homologue compositions of the samples, the most abundant of which was C21:0 *(data not shown*). In Figure 18, the total arabinoxylan (TOAX) contents in the fractions obtained from air classification process are reported.

The TOAX content in "AS IS" materials, in average, was 3.95% d.m., with a range from 4.58% d.m. (wholemeal) to 3.58% d.m. (170 G1 fraction). The hydrothermal samples showed a mean content of 3.91 % d.m. and a range from 4.50% d.m. (85 F1+F2 fraction) to 3.65% d.m. (85 G2 fraction).

Different amounts of total arabinoxylans were found between flour fractions; in particular the content decreased after hydrothermal process except for the fraction G2 that showed an increase in comparison with the as is materials.

These data are in agreement with those reported by Dharmaraj *et al*., (2011) who showed that total dietary fiber content did not significantly change after hydrothermal processing.

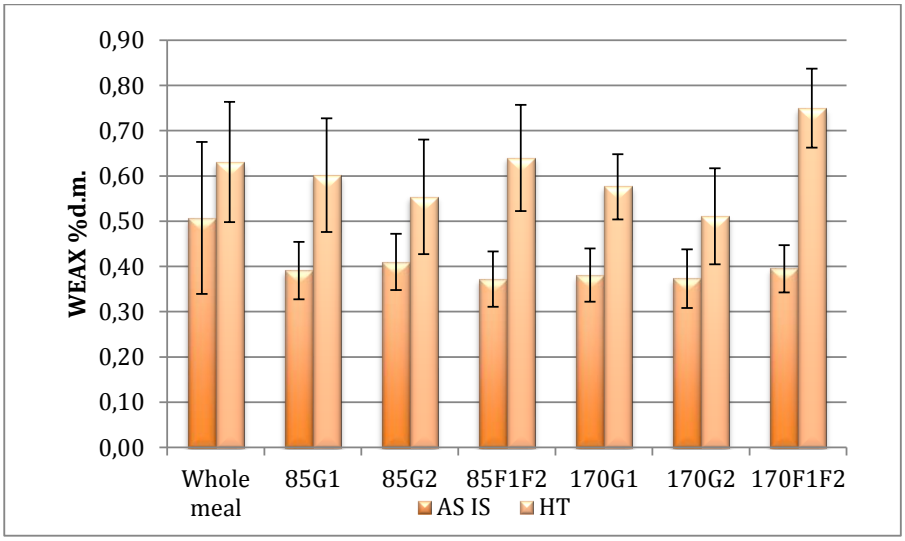
Figure 18 Influence of technological process on TOAX (total arabinoxylans) content (mean values of five durum wheat varieties). Bars represent standard deviations.



Roberto Ciccoritti

However high variation of TOAX was observed between different flour fractions in agree with Izydorczyc *et al.*, (1992) who observed a general reduction of total dietary fiber contents after milling process. WEAX content ranged from 0.51% d.m. (wholemeal) to 0.37% d.m. (85 F1+F2 fraction) in the "AS IS" grain and from 0.51% d.m. (170 G2 fraction) to 0.75% d.m. (170 F1+F2 fraction) in treated ones (Figure 19).

Figure 19 Influence of technological process on WEAX (Water extractable arabinoxylans) compounds (mean values of five durum wheat varieties). Bars represent standard deviations.



Significant differences were found between the flour WEAX contents of the "AS IS" and hydrothermal material; in fact, in all the hydrothermal materials a significant increase (about 20%) was observed. Similar results have been reported by Jaskari *et al.*, (1995) on the effect of heat treatment on oat soluble β-glucans.
The high content of arabinoxylans in fraction G2 suggested a major presence of the outer pericarp tissues in this flour fraction as described by Hemery *et al.*, (2010).

5.3.3 Semolina enrichment by micronized and air classified fractions.

The enrichment of traditional semolina was carried out with 20% of G1+G2 fraction obtained after hydrothermal process at two micronization speeds (85 and 170 Hz). The AR contents of traditional semolina was about 6.20 μg/g d.m.; after enrichment with G1+G2 fraction the contents increased about 700%, ranging from

44.84 μg/g d.m. (semolina with 20% of 85 G1+G2 fraction) to 47.02 μg/g d.m. (semolina with 20% of 170 G1+G2 fraction) as reported in Figure 20.

The different semolina samples, obtained by three cultivar under investigations, revealed similar composition of AR homologues (Table 10), while in comparison with durum wheat whole grain, the semolina AR composition showed only the homologues C19:0, C21:0 and C23:0 which were the most abundant in durum wheat whole grain.

Table 10. Homologue composition (%) determined in wholegrain and semolina samples of three durum wheat cultivars

Samples		C17:0	C19:0	C21:0	C23:0	C25:0
Meridiano	Wholegrain	0.69	11.91	65.81	15.71	5.89
	Semolina	nd	11.73	79.38	8.89	nd
Saragolla	Wholegrain	0.70	15.83	64.25	14.32	4.89
	Semolina	nd	10.67	75.13	14.20	nd
Svevo	Wholegrain	0.58	10.04	64.09	18.33	6.96
	Semolina	nd	11.24	74.35	14.41	nd
Average	Wholegrain	0.66	12.59	64.72	16.12	5.91
	Semolina	nd	11.21	76.29	12.50	nd

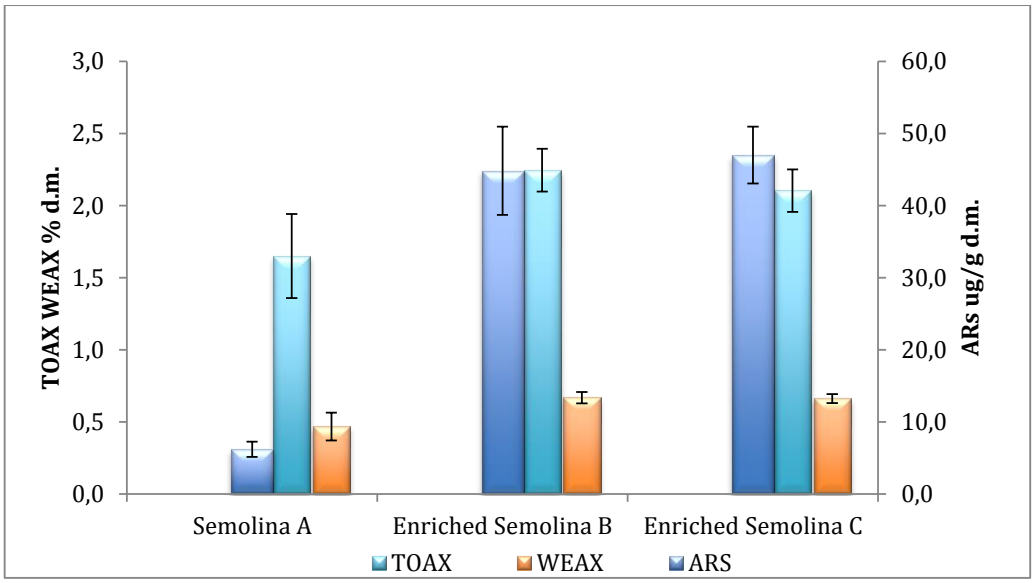
Total Arabinoxylans (TOAX) showed a range of variations between 1.65% d.m. (traditional semolina flour) and 2.25 % d.m. (semolina with 85 G1+G2 fraction) (Figure 20). In a recent study, Izydorczyk *et al*., (2005) reported that the nutritional value of noodles increased after enrichment with fiber-rich fractions derived from roller milling of hull-less barley. In particular they compared the wheat flour noodles with enriched noodles and observed much more dietary fiber in the latter (especially concerning β-glucans and arabinoxylans).

Similarly to AR also TOAX and WEAX content was highest in enriched semolina than in traditional semolina (Figure 20).

In particular WEAX showed a range of variations between 0.46% d.m. (traditional semolina) and 0.65 % d.m. (semolina with 85 G1+G2 fraction)

Roberto Ciccoritti

Figure 20 ARs (5n-alkylresorcinols), TOAX (Total arabinoxylans) and WEAX (Water extractable arabinoxylans) contents in different semolina formulations. A) 100% Semolina B) 20% 85 G1+G2 fraction C) 20% 170 G1+G2 fraction (mean values of three durum wheat varieties). Bars represent standard deviations).



These results are in agreement with those reported by Verardo *et al*. (2011), that on functional spaghetti produced by air classification of barley grain, showed that total, insoluble, and soluble fiber and β-glucan contents of the barley spaghetti were found to be greater than those of commercial whole semolina samples.

Roberto Ciccoritti

6 Conclusions

6.1 Effects of genetic and environmental variations on specific bioactive components (TOAX, WEAX, ARs, STP and AA) of the durum wheat whole grain

The aim of this research was to characterize the whole grain of a wide range of thirty durum wheat Italian varieties for the content in specific bioactive components as arabinoxylans (AX), the most abundant polysaccharide constituents of the cell wall in wheat, 5-n-alkilresorcinols (ARs), phenolic lipids with important healthy properties, free phenolic compounds with antioxidant activity (STP) and antiradical activity (AA).

By statistical analysis, the data highlight that genotype and environment are the main factors influencing the phytochemical content of whole grain and significant E and G effects were found for all analyzed traits ($p < 0.001$ and $p < 0.01$). In addition, the contribution of G x E to the total variance was much lower than that due to the separate effects (G and E), and E accounted for the highest proportion of the variation.

Considerable differences among cultivars for the bioactive compounds contents were shown. Principal component analysis bi-plots showed that in some Italian durum wheat varieties AX and AR contents were consistently higher and stable across environments. Moreover, the range of different environmental conditions recorded in this study allowed us to highlight the environmental contribution to AX and AR variability. In fact, most varieties appeared to accumulate the highest TOAX content in the environments characterized by the highest rainfall level. On the basis of these indications the environment seemed to play an important role in WEAX variability and the performance of some genotypes appeared to be strongly associated with the grown environment. On the other hand, the highest AR concentrations were associated to lowest mean temperatures and rainfall level, while high water availability during grain filling appeared to increase the accumulation of free phenols (STPC). The results emphasized the importance of G in determining the accumulation of the phytochemical components in durum wheat. Among the considered durum wheat varieties, Trionfo presented TOAX and WUAX contents higher than the mean values across all the environments; in addition the PCA allows to identify some promising varieties (Iride, Tirex, Trionfo), which are potentially

suitable for high AR or STP level in whole grain and greater stability under different environmental conditions. The graphical representation of the correspondence analysis also provided a good synthesis of the phytochemical profiles in each environment.

The results of this study will provide useful information to durum wheat scientists, regarding the relative importance of genotype and environment on the accumulation in durum wheat grains of arabinoxylans, 5-n-alkylresorcinols, total phenols and their antiradical activity. Moreover, our results clearly show that the effects of E and G x E on the levels of the examined bioactive compounds should be taken into account during the breeding programs aimed at improving the health benefit of wheat.

Main findings

- The characterization of a wide range of durum wheat Italian varieties for the content in important bioactive compounds was performed
- Significant E and G effects for all analyzed traits.
- PCA allowed us to evidence some Italian durum wheat varieties high in AX and AR contents and stability across environments
- Usefulness correspondence analysis was able to summarize the phytochemical profile of wheat.

6.2 Role of the genetic factors in determining the variations (amount and chemical composition) in phytochemicals and in the total antiradical activity of grain: ancient vs modern Triticum

A great variation in the phytochemical profiles amongst the ancient and modern wheat crops, while their scavenging capacity of DPPH radical is very similar. The adopted overall statistical model showed that the C19:0 and C21:0 homologues are the molecules with the highest discriminating power. The findings in the present thesis indicate that there is a wide variation in the AR composition in the cultivated *Triticum* species. Furthermore, evidence has been obtained that the ancient wheat crops exhibit unique bioactive phytochemical patterns, suggesting their use for production of traditional and specialty products naturally enriched with health-promoting compounds. In this context it is noteworthy that new genotypes of these

ancient wheat crops have been recently selected for their improved agronomic performance.

Main findings

- A great variation in the phytochemical profiles amongst the ancient and modern wheat crops was underlined.
- The high discriminating power of C19:0 and C21:0 homologues was found
- A wide variation in the AR composition in the cultivated *Triticum* species was evidenced.
- The unique nutraceutical properties on the basis of the ancient wheat crops exhibit their peculiar content in bioactive phytochemicals was found.

6.3 Effects of technological treatments on potential nutritional quality of raw materials for durum wheat pasta production.

The particular milling system allowed us to obtain wheat flour fractions with high bioactive compound concentrations. In addition the hydrothermal treatment increased the nutritional value of whole grain; in fact the tested conditions favored the availability of phenolic compounds with positive effects on the AR content. By these treatments durum wheat raw material for pasta making improved nutritional profile; in fact higher AR and AX concentrations in comparison with the durum wheat semolina were found in pasta formulations by enriching traditional semolina with 20% of specific flour fractions (hydrothermally treated) as a source of whole grain bioactive compounds. Additionally, significant differences among durum wheat varieties were observed confirming also the important role of raw material to maximize health benefits of whole grain products. Further the data showed that the tested milling treatment did not influence homologue composition of ARs in flour fractions. Regarding future studies, new strategies will be examined to produce raw material suitable for nutritionally improved pasta production also considering the organoleptic characteristics which could be affected by the added flour fractions.

Main findings

- A new milling system for producing durum wheat flour fractions useful to enrich semolina was tested.

- The usefulness of hydrothermal treatment for nutritional aspects of the enriched semolina was evidenced.
- Significant differences in the composition of flour fraction among the durum wheat varieties were evidenced.
- Suitable formulations for producing more healthy and nutritious foods were identified.

Roberto Ciccoritti

7 References

Abdel-Aal E.S., Rabalski I. (2008). *Bioactive compounds and their antioxidant capacity in selected primitive and modern wheat species*. The Open Agriculture Journal 2:7-14.

AACC (1995). *Approved Methods of the American Association of Cereal Chemists*. American Association of Cereal Chemists, Saint Paul, MN, USA.

AACC (2001). *Approved Methods of the American Association of Cereal Chemists*. American Association of Cereal Chemists, Saint Paul, MN, USA.

AACC (2003). *Approved Methods of the American Association of Cereal Chemists*. American Association of Cereal Chemists, Saint Paul, MN, USA.

Albersheim P., Nevins D.J., English P.D., Kan A. (1967). *A method for the analysis of sugars in plant cell-wall polysaccharides by gas liquid chromatography.* Carbohydr. Res. 5:340–345.

American Dietetic Association. (2008). *Position of the American Dietetic Association: Health implications of dietary fiber.* J. Am. Diet. Assoc. 108:1716-1731.

Andersson R., Westerlund E., Tilly A.C., Aman P. (1993). *Natural variation in the chemical composition of white flour.* J. Cereal Sci. 17:183-189.

Anderson J.W. and Hanna T.J. (1999). *Whole grains and protection against coronary heart disease: what are the active components and mechanisms?* Am J Clin Nutr. 70:307-308.

Andersson A.A.M., Kamal-Eldin A., Fras A., Boros D., Aman P. (2008). *Alkylresorcinols in wheat varieties in the HEALTHGRAIN diversity screen.* J. Agric. Food Chem. 56:9722-9725.

Andersson A.A.M., Kamal-Eldin A., Aman P. (2010). *Effects of environment and variety on alkylresorcinols in wheat in the HEALTHGRAIN Diversity Screen*. J. Agric. Food Chem 58:9299-9305.

Roberto Ciccoritti

AOAC (1995). *Official Methods of Analysis, 16th ed.* Cunniff P., eds. Association of Official Analytical Chemists, Gaithersburg, MD, USA.

Baerson S.R., Schröder J., Cook D., Rimando A.M., Pan Z., Dayan F.E., Noonan B.P., and Duke S.O. (2010). *Alkylresorcinol biosynthesis in plants.* Plant Signal Behav. 5(10):1286–1289.

Bosscher D., Van Caillie-Bertrand M., Van Cauwenbergha R., Deelstra H. (2003). *Availabilities of calcium, iron, and zinc from dairy infant formulas is affected by soluble dietary fibers and modified starch fractions.* Nutr. 19:641-645.

Bowden W.M. (1959). *The taxonomy and nomenclature of wheats, barleys, and ryes and their wild relatives.* Canadian Journal of Botany 37:657–684.

Bettge A.D., Morris C.F. (2000). *Relationships among grain hardness, pentosan fractions and end-use quality of wheat.* Cereal Chem. 77:241-247.

Bjorcka I., Ostmana E., Kristensenb M., Ansonc N.M., Priced R.K., Haenenc G.R.M.M., Havenaare R., Knudsenf K.E.B., Fridg A., Mykkanenh H., Welchd R.W. and Riccardi G. (2012). *Cereal grains for nutrition and health benefits: Overview of results from in vitro, animal and human studies in the HEALTHGRAIN project*. Trends in Food Sci. & Technol. 25:87-100.

Brand-Willams W., Cuvelier M.E., Berset C. (1995). *Use of a free radical method to evaluate antioxidant activity.* Lebensmittel- Wissenschaft and Technologie 28:25-30.

Camire M.E. (2004). *Technological challenges of whole grains*. Cereal Food World *49*:20-22.

Carbone K., Giannini B., Picchi V., Lo Scalzo R., Cecchini F. (2011). *Phenolic composition and free radical scavenging activity of different apple varieties in relation to the cultivar, tissue type and storage.* Food Chem. 127(2):493-500.

Cheng Z., Su L., Moore J., Zhou K., Luther M., Yin J.J., Yu L. (2006). *Effects of postharvest treatment and heat stress on availability of wheat antioxidants.* J. Agric. Food Chem. 54:5623-5628.

Coles G.D., Hartunian-Sowa S.M., Jamieson P.D., Hay A.J., Atwell W.A., Fulcher R.G. (1997). *Environmentally-Induced variation in starch and non-starch polysaccharide content in wheat.* J. Cereal Sci. 26:47-54.

Courtin C.M., Delcour J.A. (1998). *Physicochemical and bread-making properties of low molecular weight wheat-derived arabinoxylans.* J. Agric. Food Chem. 46:4066-4073.

Courtin C.M., Delcour J.A. (2002). *Arabinoxylans and endoxylanases in wheat flour bread-making.* J. Cereal Sci. 35:225-243.

Craeyveld V.V. (2009). *Production and functional characterization of arabinoxylan-oligosaccharides from wheat (Triticum aestivum L.) bran and psyllium (Plantago ovata Forsk) seed husk.* PhD Thesis. Faculty of Bio-ingenieurswetenschappen of the University KU Leuven, Heverlee, België

Curtis B.C. (2002). *Wheat in the world. Bread wheat improvement and production.* Curtis B.C., Rajaram S., Macpherson H.G., eds. Plant production and protection series. FAO Rome, Italy: 1-19.

Dharmaraj U., Malleshi N.G. (2011). *Changes in carbohydrates, proteins and lipids of finger millet after hydrothermal processing LWT.* Food Sci. Techn. 44(7):1636-1642.

Delcour J.A., Rouau X., Courtin C.M., Poutanen K., Ranieri R. (2012). *Technologies for enhanced exploitation of the health-promoting potential of cereals.* Trends Food Sci. & Techn. 25:78-86.

De Moura F.F., Lewis K.D., and Falk M.C. (2009). *Applying the FDA Definition of Whole Grains to the Evidence for Cardiovascular Disease Health Claims.* J. Nutr. 139:11 2220S-2226S.

Dornez E., Gebruers K., Joye I.J., De Ketelaere B., Lenartz J., Massaux C., Bodson B., Delcour J.A., Courtin C.M. (2008). *Effects of genotype, harvest year and genotype-by-harvest year interactions on arabinoxylan, endoxylanase activity and endoxylanase inhibitor levels in wheat kernels.* J. Cereal Sci. 47:180-189.

Roberto Ciccoritti

Douglas S.G. (1981). *A rapid method for the determination of pentosans in wheat flour.* Food Chem. 7:139-145.

Englyst H.N., Quigley M.E, Hudson G.J. (1994). *Determination of dietary fibre as non-starch polysaccharides with gas-liquid chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars.* Analyst 119:1497-1509.

FAO (2009). *Food and Agricultural Commodities Production*. Available from: http://faostat.fao.org/site/339/default.aspx.

FAOSTAT (2013). *Food and Agriculture Organization of the United Nations*. Available from http://faostat.fao.org.

FAO/WHO (2003). *Diet, nutrition and the prevention of chronic diseases*. Available from http://www.who.int/dietphysicalactivity/publications/trs916/en/index.html).

Fausch H., Kuindig W., Neukom H. (1963). *Ferulic acid as a component of a glycoprotein from wheat flour.* Nature 199:287-293.

Ferrari B., Finocchiaro F., Stanca A.M., Gianinetti A. (2009). *Optimization of air classification for the production of β-glucan-enriched barley flours.* J. Cereal Sci. 50:152-158.

Finnie S.M., Bettge A.D, Morris M.F. (2006). *Influence of cultivar and environment on water-soluble and water-insoluble arabinoxylans in soft wheat.* Cereal Chem. 83:617-623.

Flight I. and Clifton P. (2006). *Cereal grains and legumes in the prevention of coronary heart disease and stroke: a review of the literature.* Eur. J. Clin. Nutr. 60:1145-1159.

Frederix S. (2004). *Arabinoxylans and endoxylanase functionality in wheat flour gluten-starch separation.* Laboratorium voor Levensmiddelenchemie en-biochemie Publications.

Roberto Ciccoritti

Frontela C., Ros G., Martínez C. (2011). *Phytic acid content and "in vitro" iron, calcium and zinc bioavailability in bakery products: The effect of processing*. J. Cereal Sci. 54:173-179.

Gajda A., Kulawinek M., Kozubek A. (2008). *An improved colorimetric method for the determination of alkylresorcinols in cereals and whole-grain cereal products.* J. Food Comp. Anal. 21(5):428-434.

Garcia A., Otto B., Reich S.C., Weickert M.O., Steiniger J., Machowetz A., Rudovich N.N., Katz N., Speth M., Meuser F. (2007). *Arabinoxylan consumption decreases postprandial serum glucose, serum insulin and plasma total ghrelin response in subjects with impaired glucose tolerance.* Eur. J. Clin. Nutr. 61:334-341.

Gebruers K., Dornez E., Boros D., Fras A., Dynkowska W., Bedo Z., Rakszegi M., Delcour J.A., Courtin C.M. (2008). *Variation in the content of dietary fiber and components thereof in wheats in the HEALTHGRAIN diversity screen.* J. Agric. Food Chem. 56:9740-9749.

Gebruers K., Dornez E., Bed Z., Rakszeg M., Fras A., Boros D., Courtin C.M., Delcour J.A. (2010). *Environment and genotype effects on the content of dietary fiber and ist components in wheat in the HEALTHGRAIN diversity screen.* J. Agric. Food Chem. 58:9353-9361.

Grootaerta C., Delcourb J.A., Courtinb C.M., Broekaertb W.F., Verstraetea W. and Van de Wiele T. (2007). *Microbial metabolism and prebiotic potency of arabinoxylan oligosaccharides in the human intestine.* Trends in Food Science & Technology 18:64-71.

Gunenc A., HadiNezad M., Tamburic-Ilincic L., Mayer P.M., Hosseinian F. (2013). *Effects of region and cultivar on alkylresorcinols content and composition in wheat bran and their antioxidant activity*. J. Cereal Sci. 57:405-410.

Hartunian S.M., and. White P.J. (1992). *Characterisation of starch isolated from oat groats with different amount of lipid*. Cereal Chem. 69:521–527.

Roberto Ciccoritti

HealthGrains (2011). *The Grains & Legumes Health Report*. Available from "http://www.healthgrain.org."

Heinemann R.J.B., Fagundes P.L., Pinto E.A., Penteado M.V.C., Lanfer-Marquez U.M. (2005). *Comparative study of nutrient composition of commercial brown. parboiled and milled rice from Brazil.* Journal of Food Composition and Analysis 18:287-296.

Hemery Y., Rouau X., Lullien-Pellerin V., Barron C., Abecassis J. (2007). *Dry processes to develop wheat fractions and products with enhanced nutritional quality*. J. Cereal Sci. 46:327-347.

Hemery Y., Lullien-Pellerin V., Rouau X., Barron C., Abecassis J., Samson M.F., Åman P., Von Reding W., Spoerndli C., Barron C. (2009). *Biochemical markers: efficient tools for the assessment of wheat grain tissue proportions in milling fractions.* J. Cereal Sci. 49:55-64.

Hemery Y., Holopainen U., Lampi A-M., Lehtinen P., Nurmi T., Piironen V., Edelmann M., Rouau X. (2010). *Potential of dry fraction of wheat bran for the development of food ingredient, part II: Electrostatic separation of particles.* J. Cereal Sci. 53(1):9-18.

Hidalgo A., Brandolini A., Gazza L. (2008). *Influence of steaming treatment on chemical and technological characteristics of einkorn (Triticum monococcum L. ssp. monococcum) wholemeal flour*. Food Chem. 111:549-555.

Hong B.H., Rubenthaler G.L., Allan R.E. (1989). *Wheat pentosans. I. Cultivar variation and relationship to kernel hardness.* Cereal Chem. 66:369-373.

INRAN (2003). *Linee guida per una sana e corretta alimentazione italiana.* Available from: "www.inran.it /648/linee_guida.html"

Izydorczyk M.S., Lagasse S.L., Hatcher D.W., Dexter J.E., and Rossnagel B.G. *(2005). The enrichment of Asian noodles with fiber-rich fractions derived from roller milling of hull-less barley.* J. Sci. Food Agric. 85:2094-2104.

Roberto Ciccoritti

Izydorczyk M.S. and Biliaderis C.G. (1992). *Effect of molecular size on physical properties of wheat arabinoxylan.* J. Agric. Food Chem. 40:561-568.

Jacobs D., Anderson F.L., and Blomhoff R. (2007). *Wholegrain consumption is associated with a reduced risk of noncardiovascular, noncancer death attributed to inflammatory disease in the Iowa Women's Health Study*. Am. J. Clin. Nutr. 85:1606-1614.

Jaskari J., Henriksson K., Nieminen A., Suortti T., Salovaara H., and Poutanen K. (1995). *Effect of Hydrothermal and Enzymic Treatments on the Viscous Behavior of Dry and Wet Milled Oat Brans*. Cereal Chem. 72:625-631.

Jones J. (2007). *Mining wholegrains for functional components*. Food Science and Technology Bulletin 4(7):67-86.

Jones J.M., Engleson J. (2010). *Whole grains: Benefits and challenges*. Annu. Rev. Food Sci. Technol. *1*:19-40.

Kabel M.A., Schols H.A., Voragen A.G.J. (2002). *Complex xylo-oligosaccharides identified from hydrothermally treated Eucalyptus wood and brewery's spent grain*. Carbohydrate Polymers 50(2):191-200.

Kamal-Eldin A., Pouru A., Eliasson C., Aman P. (2000). *Alkylresorcinols as antioxidants: hydrogen donation and peroxyl radical-scavenging effects.* J. Sci. Food Agri. 81:353-356.

Kellogg E.A. (1998). *Relationships of cereal crops and other grasses*. Proceedings of the National Academy of Sciences of the United States of America PNAS. 95(5):2005-2010.

Kim J.H., Tanhehco E.J., NG P.K.W. (2006). *Effect of extrusion conditions on resistant starch formation from pastry wheat flour.* Food Chem. 99:718-723.

Kimura. T., Matsuda. J., Ikeuchi. Y. and Yoshida T. (1976). *Basicstudies on parboiled rice (Part II): Effect of processing conditionson the rate of gelatinization of parboiled rice.* J. Jpn. Soc. Agric. Machinery 38(1):47-52.

Roberto Ciccoritti

Knodler M., Most M., Schieber A., Carle R. (2010). *A novel approach to authenticity control of whole grain durum wheat (Triticum durum desf.) flour and pasta, based on analysis of alkylresorcinol composition.* Food Chem. 118:177-181.

Kozubek A., Demel R.A. (1981). *The effect of 5-alk(en)yl)resorcinols from rye on membrane structure.* Biochimica Biophysica Acta 642:242-251.

Kozubek A., Nietubyc M., Sikorski A.F. (1992). *Modulation of the activity of the membrane enzymes by cereal grain resorcinolic lipids.* Z Naturforsch 47:41-46.

Kozubek A., Tyman J.H.P. (1999). *Resorcinolic lipids, the natural non-isoprenoid phenolic amphiphiles and their biological activity.* Chem. Rev. 99(1):1-25.

Kuijsten A., Arts I., Van't Heer P., and Hollman P. (2005). *The relative bioavailability of enterolignans in humans in enhanced by milling and crushing of flaxseed.* J. Nutr. 135:2812-2816.

Kushi L.H., Meyer K.A., and Jacobs D.R. (1999). *Cereals, legumes, and chronic disease risk reduction: evidence from epidemiologic studies*. Am. J. Clin. Nutr. 70(3):451S-458.

Labat E., Rouau X., Morel M.H. (2002). *Effect of flour water-extractable pentosans on molecular associations in gluten during mixing.* LWT-Food Sci. Technol. 35:185-189.

Lai V.M.F., Shin L, Heisien H.W., Haan C.H. (2007). *Non-starch polysaccharide compositions of rice grains with respect to rice variety and degree of milling.* Food Chem. 101:1205-1210.

Landberg R., Deyb E.S., Franciscob J.D.C., Åmana P., Kamal-Eldina A. (2007). *Comparison of supercritical carbon dioxide and ethyl acetate extraction of alkylresorcinols from wheat and rye.* Journal of Food Composition and Analysis 20:534–538.

Roberto Ciccoritti

Landberg R., Andersson A.A.M., Aman P., Kamal-Eldin A. (2009). *Comparison of GC and colorimetry for the determination of alkylresorcinol homologues in cereal grains and products.* Food Chem. 113:1363-1369.

Legge N° 580. (1967) e successive modifiche (*2001). Disciplina per la lavorazione e commercio dei cereali, degli sfarinati, del pane e delle paste alimentari.* Gazzetta Ufficiale della Repubblica Italiana n°189 del 29 luglio 1967 e *n. 187* n°. 117 del 22/05/2001.

Lempereur I., Rouan X., Abecassis J. (1997). *Genetic and agronomic variation in arabinoxylan and ferulic acid contents of durum wheat (Triticum durum L.) grain and its milling fractions.* J. Cereal Sci. 25:103-110.

Li S., Morris C.F., Bettge A.D. (2009). *Genotype and environment variation for arabinoxylan in hard winter and spring wheats of the U.S. Pacific Northwest.* Cereal Chem. 86:88-95.

Lindsay D.G. (2005). *Nutrition, hormetic stress and health*. Nutr. Res. Rev. 18:249-258.

Liu R.H. (2007). *Whole grain phytochemicals and health*. J. Cereal Sci. 46:207-219.

Lopez H.W., Levrat M.A., Guy C., Messager A., Demigné C., Rémésy C., Dongowski G. (1999). *Effects of soluble corn bran arabinoxylans on cecal digestion, lipid metabolism, and mineral balance (Ca, Mg) in rats* J. Nutr. Biochem. 10(9):500-509.

Manning T.S., Gibson G.R., (2004). *Prebiotics.* Best Practice & Research Clinical Gastroenterology 18:287-298.

Mares D.J., Stone B.A. (1973). *Studies on wheat endosperm. I. Chemical composition and ultrastructure of the cell walls*. Aust. J. Biol. Sci. 2:793-812.

Martinant J.P., Billot A., Bouguennec A., Charmet G., Saulnier L., Branlard G. (1999). *Genetic and environmental variations in water-extractable arabinoxylans content and flour extract viscosity.* J. Cereal Sci. 30:45-48.

Roberto Ciccoritti

McKevitt C., Redfern J., Mold F. and Wolfe C. (2004). *Qualitative studies of stroke: a systematic review*. Stroke.35:1499-1505.

Mercader J., Asmeron Y., Bennett T., Raja M., and Skinner A. (2009). *Initial excavation and dating of Ngalue Cave - A middle stone age site along the Niassa Rift, Mozambique*. J. Hum. Evol. 57:63-74.

Menga V., Fares C., Troccoli A., Cattivelli G., Baiano A. (2010). *Effects of genotype, location and baking on the phenolic content and some antioxidant properties of cereal species.* Int. J. Food Sci. Tech. 45:7-16.

Michalska A., Ceglinska A., Zielinski H. (2007). *Bioactive compounds in rye flours with different extraction rates*. Eur. Food. Res. Technol. 225:545-551.

Moore J.C. (2007). *Enhancing the availability of natural antioxidants in wheat-based food ingredients and food products through improved post-harvest treatments and processing conditions* PhD Thesis.: Faculty of the Graduate School of the University of Maryland, College Park.

Moore J., Yu L.L. (2008). *Methods for antioxidant capacity estimation of wheat and wheat-based food products.* In: Yu L., Mac Graw-Hill, eds. *Wheat Antioxidant*. New York: pp 147-150.

Mpofu A., Saperstein H.D., Beta T. (2006). *Genotype and environmental variation in phenolic content, phenolic acid composition and antioxidant activity of hard spring wheat.* Journal of Agricultural and Food Chem. 54:1265-1270.

Nystrom L., Lampi A.M., Andersson A.A.M, Kamal-Eldin A., Gebruers K., Courtin C.M., Delcour J.A., Li L., Ward J.L., Fras A., Boros D., Rakszegi M., Bedo Z., Shewry P.R., Piironen V. (2008). *Phytochemicals and dietary fiber components in rye varieties in the Healthgrain diversity screen.* J. Agric. Food Chem. 56(21):9758-9766.

NIIR Board of Consultants & Engineers. (2006). *Wheat, Rice, Corn, Oat, Barley and Sorghum Processing Handbook (Cereal Food Technology).* Publisher: Asia Pacific Business Press Inc.

Roberto Ciccoritti

Nocente F., Ciccoritti R., Sereni L., Matere A., Sgrulletta D., Pasquini M. (2012). *Protective effect of bioactive compounds extracted from wheat whole grain against different FHB causal agent.* Abstract of International MPU Workshop Plant protection for the quality and safety of the mediterranean diet, p. 36.

Ogbonnaya C., Friday J.O. (2009). *Response of Nutritional Contents of Rice (Oryza sativa) to Parboiling Temperatures*. American Eurasian Journal of Sustainable Agriculture 3(3):381-387.

Ordaz-Ortiz J.J., Saulnier L. (2005). *Structural variability of arabinoxylans from wheat flour. Comparison of water-extractable and xylanase-extractable arabinoxylans*. J. Cereal Sci. 42:119–125.

Panatta G.B. (1997). *Cereali e patate.* F. Fidanza & G. Liguori, eds. Nutrizione Umana. Idelson, Napoli Italy. 268-289.

Perlin A.S. (1951). *Structure of the soluble pentosans of wheat flours*. Cereal Chem. 28:328-393.

Poutanen K. (2012). *Past and future of cereal grains as food for health Trends*. Food Sci. Tech. 25:58-62.

Prior R.L., Wu X., Schaich K. (2005). *Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements.* J. Agri. Food Chem. 53:4290-4302.

Rejman J., Kozubek A. (2004). *Inhibitory Effect of Natural Phenolic Lipids upon NAD-Dependent Dehydrogenases and on Triglyceride Accumulation in 3T3-L1 Cells in Culture*. J. Agric. Food Chem. 52(2):246-250.

Redaelli R., Sgrulletta D., Scalfati G., De Stefanis E. (2006). *Development of naked-oat products with suitable nutritional properties for improving health.* Tecn. Mol. Intern. 57, 5/A, 1-7.

Roberto Ciccoritti

Ross A.B. (2012). *Present status and perspectives on the use of alkylresorcinols as biomarkers of wholegrain wheat and rye intake.* J. Nutr. Metabolism. http://dx.doi.org/10.1155/2012/462967. Article ID 462967.

Ross A.B., Shepherd M.J., Schupphaus M., Sinclair V., Alfaro B., Kamal-Eldin A., Aman P. (2003). *Alkylresorcinols in cereals and cereal products.* J. Agric. Food Chem. 51:4111-4118.

Ross A.B., Kamal-Eldin A., Aman P. (2004). *Dietary alkylresorcinols: absorption, bioactivities, and possible use as biomarkers of whole-grain wheat and rye rich foods*. Nutr. Rev. 62(3):81-95.

Sanchez-Moreno C., Larrauri J.A., Saura-Calixto F. (1998). *A procedure to measure the antiradical efficiency of polyphenols.* J. Sci. Food Agric. 76:270-276.

Saulnier L., Peneau N., Thibault J.F. (1995). *Variability in grain extract viscosity and water soluble arabinoxylan content in wheat.* J. Cereal Sci. 22:259-264.

Saura-Calixto F., Perez-Jimenez J., Goni I. (2009). *Contribution of cereals to dietary fiber and antioxidant intakes: toward more reliable methodology*. J. Cereal Sci. 50:291-294.

Scalbert A., Williamson G. (2000). *Dietary intake and bioavailability of polyphenols*. J. Nutr. 130:2073-2085.

Schlemmer U., Frolich W., Prieto R., and Grases F. (2009). *Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis.* Mol. Nutr. Food Res. 53(S2):S330-S375.

Schultz E. (2011). *Dietary Fiber.* Available from "http:www.effieschultz.com".

Shewry P. R. and Ward J.L. (2009). *Analysis of Bioactive Compounds in Small Grain Cereals.* Healthgrain methods ed. AACC International. 4:25-40.

Shewry P.R. (2009). *The HEALTHGRAIN programme opens new opportunities for improving wheat for nutrition and health*. Nutr. Bull. 34:225-231.

Roberto Ciccoritti

Shewry P.R., Piironen V., Lampi A.M., Edelmann M., Kariluoto S., Nurmi T., Nyström L., Ravel C., Charmet G., Andersson A.A.M., Åman P., Boros D., Gebruers K., Dornez E., Courtin C.M., Delcour J.A., Rakszegi M., Bedo Z., Ward J.L. (2010). *The HEALTHGRAIN wheat diversity screen: effects of genotype and environment on phytochemicals and dietary fiber components.* J. Agric. Food Chem. 58:9291-9298.

Sissons M. (2008). *Role of durum wheat composition on the quality of the pasta and bread.* Food 2: 75-90.

Slavin J.L., Jacobs D., Marquart L., Wiener K. (2001). *The role of whole grain in disease prevention.* J. Am. Diet. Assoc. 101:780-785.

Slavin J. (2003). *Why wholegrains are protective: biological mechanisms.* Proceedings of the Nutrition Society 62(1):129-134.

Slavin J. (2004). *Whole grains and human health*. Nutr Res Rev 17: 99-110.

Slavin J., Jacobs D., and Marquart J. (2001). *Grain processing and nutrition.* Critical Reviews in Biotechnology 21(1):49-66.

Stasiuk M., Bartosiewicz D., Kozubek A. (2008). *Inhibitory effect of some natural and semisynthetic phenolic lipids upon acetylcholinesterase activity.* Food Chem. 108:996-1001.

Stasiuk M., Kozubek A. (2010). *Biological activity of phenolic lipids.* Cell. Mol. Life Sci. 67:841-860.

Steffen L.M., Kroenke C.H., Yu X., Pereira M.A., Slattery M.L., Horn L.V., Gross M.D. and Jacobs D. (2005). *Associations of plant food, dairy product, and meat intakes with 15-y incidence of elevated blood pressure in young black and white adults: the Coronary Artery Risk Development in Young Adults (CARDIA)Study.* Am. J. Clin. Nutr. 82:1169-1177.

Subaric D., Babic J., Ackar D., Pilizota V., Kopjar M., Ljubas I., Ivanoska S. (2011). *Effect of galactomannan hydrocolloids on gelatinization and retrogradation of tapioca and corn starch.* Croat. J. Food Sci. Technol. 3(1):26-31.

Roberto Ciccoritti

Suzuki H., Ueda T., Ichikawa T. and Ito H. (2003). *Androgen receptor involvement in the progression of prostate cancer*. Endocr. Relat. Cancer 10:209-216.

Trichopoulou A. and Lagiou P. (1997). *Healthy Traditional Mediterranean Diet: An Expression of Culture, History, and Lifestyle.* Nutrition Reviews 55(11):383-389.

Turner M.A., Soh C.H.N., Ganguli N.K., Sissons M.J. (2008). *A survey of water-extractable arabinopolymers in bread and durum wheat and the effect of water-extractable arabinoxylan on durum dough rheology and spaghetti cooking quality*. J. Sci. Food Agric. 88:2551-2555.

Van Laere K.M.J., Hartemink R., Bosveld M., Schols H.A., and Voragen A.G.J. (2000). *Fermentation of Plant Cell Wall Derived Polysaccharides and Their Corresponding Oligosaccharides by Intestinal Bacteria*. J. Agric. Food Chem. 48(5):1644-1652.

Verardo V., Gómez-Caravaca A.M., Messia M.C., Marconi E., Caboni M.F. (2011). *Development of functional spaghetti enriched in bioactive compounds using barley coarse fraction obtained by air classification.* J Agric Food Chem. 59(17):9127-34.

Wang L.Z. and White P.J. (1994). *Structure and Physicochemical Propertis of Starch from Oats with Different Lipid Contents*. AACC. 71:443-450.

Ward J.L., Poutanen K., Gebruers K., Piironen V., Lampi A.M., Nyström L., Andersson A.A.M., Åman P., Boros D., Rakszegi M., Bedő Z. and Shewry P.R. (2008). *The HEALTHGRAIN Cereal Diversity Screen: Concept, Results, and Prospects.* J. Agric. Food Chem. 56:9699-9709.

Wattenberg L.W. (1985). *Chemoprevention of cancer.* Cancer Res. 45: 1-8.

Williams P. (2010). *The Grains & Legumes Health Report*. Available from "http://www.healthgrain.org."

Wong C.E., Li Y., Labbe A., Guevara D., Nuin P., Whitty B., Diaz C., Golding G.B., Gray R.G., Weretilnyk E.A., Griffith M., Moffatt B.A. (2006). *Transcriptional profiling implicates novel interactions between abiotic stress and hormonal*

responses in Thellungiella, a close relative of Arabidopsis. Plant Physiol. 140:1437-1450.

Young R., Gomez M.H., McDonough C.M., Waniska R.D., Rooney L.W. (1993). *Chages in Sorghum Starch During Parboiling*. American Association of Cereal Chemists inc. 70(2):179-183.

Yu L., Haley S., Perret J., Harrys M. (2004). *Comparison of wheat flours grown at different locations for their antioxidant properties.* Food Chem. 86(1): 11-16.

Zarnowski R., Kozubek A. (2002). *Resorcinolic lipids as natural biofungicide*. In: Dehne, H.W., Gisi, U., Kuck, K.H., Russell, P.E., Lyr, H., eds. *Modern Fungicides and Antifungal Compounds* III. AgroConcept GmbH, Bonn, Germany: 337-347.

Zhou. J.R., Mukherjee. P., Gugger. E.T., Tanaka. T., Blackburn. G.L., & Clinton S.K. (1998). *The inhibition of murine bladder tumorigenesis by soy isoflavonesvia alterations in the cell cycle. apoptosis. and angiogenesis*. Cancer Res. 58:5231-5238.

Zielinski H., Kozlowska H. (2000). *Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions.* J. Agric. Food Chem. 48:2008-2016.

Roberto Ciccoritti

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