TOTAL POLYPHENOL CONTENT AND ANTIOXIDANT PROPERTIES OF SOLINA (*TRITICUM AESTIVUM* L.) AND DERIVATIVES THEREOF

A. DURAZZO*, G. CASALE, V. MELINI, G. MAIANI and R. ACQUISTUCCI
Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - Centro di ricerca per gli alimenti e la nutrizione (CREA-AN), Via Ardeatina 546, 00178 Roma, Italy
*Corresponding author. Tel.: +39 0651494499; fax: +39 0651494550
E-mail address: alessandra.durazzo@crea.gov.it

ABSTRACT

This study aims to characterize grains and derivatives of Solina, an Italian traditional winter soft wheat (*Triticum aestivum* L.). Total polyphenol content and antioxidant properties were investigated in grains, whole flours and bread, both in aqueous-organic extracts and residues.
Results showed the important contribution of hydrolysable polyphenols, isolated in the residue of the aqueous-organic extract, to antioxidant properties (over 90% in grains and derivatives), and the key role played by milling and baking in antioxidant properties. Moreover, the analysis of potentially bioactive antioxidants remaining in the residues showed to be required for a comprehensive determination of antioxidant capacity in cereals. The study highlights that Solina represents a valuable Italian traditional wheat cultivar with proved antioxidant properties.

Keywords: antioxidants, Total Polyphenol Content (TPC), traditional foods, soft wheat Solina, whole wheat flours, bread

1. INTRODUCTION

Nowadays the traditional character of foodstuffs is a value-adding characteristic which contributes to defining quality thereof and is also often associated with positive health benefits. Traditional foods, in fact, not only play an important role in the cultural and nutritional food patterns of every country because their cultivation or preparation have been passed down from generation to generation, and/or because they have been consumed locally for years and become part of daily life in communities; they are often considered healthy and wholesome and are also often associated with positive health benefits because of their chemical profile.
Solina is a traditional Italian winter soft wheat (*Triticum aestivum* L.), typical of the province of L'Aquila, in the Abruzzo region, within the National Park of Gran Sasso. Its cultivation in Abruzzo dates back to 1500 a.C. and there is evidence of a strong connection between this variety and the life of people from Abruzzo who keep appreciating it for the sensory properties it provides to bakery products. It is grown in mountainous areas and shows a high adaptability to marginal areas, as well as resistance to cold. That ancient cultivar was, in fact, defined by last century agronomists "*Wheat hybernum*". Solina also shows a high stability in yields, and according to agronomists it is also compatible with organic farming methods because it does not require a large amount of nitrogen; moreover it is able to tolerate and compete strongly with weeds for its size and tillering capacity (PORFIRI *et al*., 2001).
In this study, grains of the Solina cultivar, whole flours and bread samples thereof were examined in order to evaluate the phenolic components and the antioxidant properties of this traditional crop.
Some technological and chemical parameters of grains were also considered, so as to identify a comprehensive portrait of this Italian wheat cultivar.
According to literature, grains, in particular whole grains, provide a wide range of nutrients and phytochemicals that work in synergy to maintain human health (LIU, 2007; OKARTER and LIU, 2010). Whole grain consumption has been associated with reduced risk of chronic diseases, such as cardiovascular diseases and cancer (SCHATZKIN *et al*., 2007; MELLEN *et al*., 2008; AUNE *et al*., 2013). These beneficial properties have been rediscovered by consumers and producers (ARVOLA *et al*., 2007).
As the content of bioactive molecules is affected by genetic and growing conditions in grains as well as by processing conditions in foods (SLAVIN *et al*., 2000), samples of Solina grains, flours and bread formulated with this soft wheat cultivar were studied.

2. MATERIALS AND METHODS

2.1. Sampling

Analysis were performed on grain, whole wheat flour and bread samples. Grains were supplied by three farms located in three different spots of the same area: Castelvecchio Subequo, Capestrano and Introdacqua. According to sample location they were labeled as Solina 1, Solina 2 and Solina 3, collected respectively at Castelvecchio Subequo, Capestrano and Introdacqua.
The grains of each collected sample were divided in two aliquots. An aliquot was milled by a water cooled mill (Janke & Kunkel IKA Labortechnik, Staufen, Germany) and stored at 4°C until chemical analyses were performed. An aliquot was tempered for 24 h to 15.5% moisture content, then milled in an experimental mill MLU-202 Bühler (Switzerland)

equipped with three breaks and three reduction rolls and six screens to obtain whole mill flours.
Solina bread was also studied. Five loaves of bread (four long loaves and one round loaf), produced according to a traditional bread making process, were purchased in bakeries located in the same area of grain collection. They were coded as reported in Table 1.

Table 1. Code, description and some chemical parameters of bread samples*.

Code	Description	Composition		
		Moisture (%)	Protein (%)	Ash (%)
A	Bread long loaf (900 g)	9.9	11.5	1.90
B	Bread long loaf (1660 g)	7.8	11.2	2.74
C	Bread round loaf (900 g)	10.8	12.5	2.96
D	Bread long loaf (810 g)	11.0	11.6	2.65
E	Bread long loaf (1700 g)	10.4	11.6	1.81

*Data are expressed as mean of replicate measurements (n=3); differences within measurements were in the range reported in the method.

2.2. Chemicals and standards

Common reagents and standards were purchased from Sigma–Aldrich Srl (Milan, Italy), Extrasynthèse (Genay, France), Carlo Erba (Milan, Italy) and BDH Laboratory Supplies (Poole, UK) and their purity degree was chosen according to the analysis to be performed. Double-distilled water (Millipore, Milan, Italy) was used throughout the study.

2.3 General chemical analysis

Grain quality was determined after removing impurities, i.e., broken grains, heat-damaged grains, shriveled grains, vegetable impurities, vitreous grains, on 100 g seeds by visual evaluation. Test weight (TW), thousand kernel weight (1,000 KW) and kernel hardness (KH) were assessed. TW was determined by a Shopper chondrometer equipped with 250 mL cylinder. 1,000 KW was determined by counting and weighing 1,000 kernels, while kernel hardness (KH), diameter and moisture of grains were determined by the SKCS 4100 apparatus (Perten Instruments, Sweden) according to the standard method AACC 55-31 (2003).
Total proteins were determined by Kjeldahl method according to ICC standard method No 105/2 (2003) using 5.70 as specific conversion factor. Ash content was determined on the inorganic residue remaining after the incineration of the sample in a muffle furnace at the temperature of 900°C according to the ICC method No 104/1 (2003), while moisture was determined according to ICC standard method No 110/1 (2003). Moisture is reported as g/100 g fresh weight (f. w.), while both protein and ash content are reported as g/100 g dry matter (d. m.).

2.4. Sample extraction for evaluation of Total Polyphenol Content (TPC) and antioxidant properties

Total polyphenols were extracted as described by DURAZZO *et al*. (2013), with some modifications. Extractable polyphenols were determined on aqueous-organic extracts, while non extractable polyphenols were determined on solid residues of aqueous-organic extraction. In details, some non-extractable polyphenols, i.e. hydrolysable polyphenols, were isolated and determined following a specific and suitable acid hydrolysis as reported below.

Extractable polyphenols

About 3.0 g, 3.5 g, 4.5 g of grains, flours and bread, respectively, were placed in a test tube and added with 20 mL of acid methanol/water (50:50 v/v, pH= 2). Tubes were swirled at room temperature for 3 min, then mildly shaken for 1 h in a water bath at room temperature as well. Tubes were centrifuged at 2500 x g for 10 min, and the supernatant was recovered. Then, 20 mL acetone/water (70:30, v/v) were added to residue and the extraction repeated as reported above. Methanolic and acetonic extracts were combined and centrifuged at 2800 x g for 15 min. The resulting mixture was then used for the determination of total polyphenol content and antioxidant properties.

Hydrolysable polyphenols

The residue left after the above described extraction was dried in a ventilated oven at 25°C. 250 mg, 300 mg, 400 mg of grain, flour and bread residue, respectively, were mixed with 20 mL of methanol and 2 mL of sulfuric acid (18 M). Samples were gently stirred for 1 min and shaked at 85°C for 20 h in a water bath.
Samples were then centrifuged (2500 x g for 10 min), and the supernatant was recovered. After two washings with minimum volume of distilled water and re-centrifuging as necessary, the final volume was taken up to 50 mL. The tube was centrifuged at 2800 x g for 20 min and the resulting supernatant was used for the determination of total polyphenol content and antioxidant properties.

2.5. Determination of Total Polyphenol Content (TPC)

The TPC was determined using the Folin-Ciocalteau procedure (SINGLETON et al., 1999). Briefly, appropriate dilutions of extracts were oxidised with Folin-Ciocalteau reagent, and the reaction was neutralised with sodium carbonate. The absorbance of the resulting blue colour solution was measured at 760 nm against an appropriate blank after 2 h of reaction at room temperature at dark. Gallic acid was used as standard.

2.6. Antioxidant properties evaluation

The determination of antioxidant properties was carried out by FRAP (Ferric Reducing Antioxidant Power) assay according to methods of BENZIE and STRAIN (1996) and PULIDO *et al*. (2000) and by using a Tecan Sunrise® plate reader spectrophotometer. The method is based on the reduction of Fe^{3+}-TPTZ (2,4,6-tripyridyl-s-triazine) complex to ferrous at low pH.

2.7. Statistical Analysis

All analyses were performed in triplicate. Data are presented as mean±standard deviation (s.d.).
Data obtained by Official Methods are presented as mean. Statistica for Windows (Statistical package; release 4.5; StatSoft Inc., Vigonza PD, Italy) was used to perform One-way Analysis of Variance (ANOVA). Significant differences between grain and flours of the same origin were evaluated by the Student's t test.

3. RESULTS AND DISCUSSIONS

The inspection of grain impurities in the samples is an important control procedure necessary to verifying wheat quality and it is a preliminary step prior to the milling phase. The percentage of ripe grains were higher than 95% in all samples. This confirms the good quality of analysed grains.
The values obtained for the main physical parameters (kernel hardness, test weight, thousand kernel weight, diameter) and the chemical composition (moisture, protein, ash) of grain samples are reported in Table 2. As concerns physical parameters, no significant differences were found among grain samples. The values obtained for TW (74.1 - 80.2 Kg/hL), 1,000 KW (>43 g) and grain diameter (2.11-2.47 mm) show that kernels are wholesome, sound, not sprout damaged.

Table 2. Physical and chemical parameters of grains*.

Sample	KH	TW (kg/hL)	1,000 KW (g)	Diameter (mm)	Moisture (g/100 g)	Protein (g/100 g d.m.)	Ash (g/100 g d.m.)
SOLINA 1	19.70	74.1	44.6	2.47	12.4	13.1	2.04
SOLINA 2	16.69	80.2	54.4	2.46	12.6	11.9	1.75
SOLINA 3	33.70	79.0	43.4	2.11	12.4	13.7	2.00

*Data are expressed as mean of replicate measurements (n=3); differences within measurements were in the range reported in the method.

As regards kernel hardness, the data reported in Table 2 confirm our samples belong to the "soft" species and their variation may depend on the different growing locations. According to POMERANZ and WILLIAMS (1990), in fact, the main factors that affect the grain hardness are growing location (soil type, elevation, planting type, irrigation, fertilizers and cultivation practice), growing season (precipitation and temperature during maturation and post ripening), storage conditions, protein content, moisture and kernel size. Regarding the main chemical parameters, significant differences were observed: Solina 2 showed the lowest protein and ash content. Those data show, therefore, that environmental and growing factors not only influence hardness, but also chemical parameters such as protein and ash content.
In Table 3, total polyphenol content (TPC) and antioxidant properties (FRAP) are reported for grains and whole wheat flours. Values refer to aqueous-organic extracts and residues. In grains, TPC values ranged between 165.57 and 183.75 mg/100g d.m. in aqueous-organic

extracts, and between 1084.42 and 1325.32 mg/100g d.m. in residues, whereas the range for FRAP values was 6.34-6.60 μmol/g d.m. in aqueous-organic extracts and 85.06-109.12 μmol/g d.m. in residues. TPC and FRAP values in aqueous-organic extracts showed no differences between the grain samples supplied by the three farmers. On the other hand, as to the residue, the TPC resulted significantly different in the grain samples and the highest value was obtained for Solina 1; the values for antioxidant properties in Solina 1 were higher than those obtained for Solina 2 and Solina 3 that were, at their turn, comparable. As reported by several authors (ADOM *et al*., 2003; CARCEA *et al*., 2009) differences in the bioactive molecule distribution and antioxidant properties in wheat, as well as in foodstuffs in general, could be related to genetic factors, agronomic practices and environmental conditions. It is interesting to notice that in grains the FRAP values in residues are about 15 fold higher than in aqueous-organic extracts. This trend was also confirmed by the data obtained for TPC. The hydrolysable polyphenols, isolated in residue, belong to several classes of phenolic compounds, i.e. hydrolysable tannins, phenolic acids and hydroxycinnamic acids, and consist on ferulic acid, caffeic acid, sinapic acid, etc. These phenols are linked to carbohydrates and protein by covalent bonds, hydrogen bonds and/or hydrophobic interactions and they are released from food matrix after a strong acid hydrolysis (PÉREZ JIMÉNEZ and TORRES, 2011; PÉREZ-JIMÉNEZ *et al*., 2013). These compounds represent a significant fraction in some groups of foods, among which cereals and have appreciable antioxidant properties and possibly specific health properties. However, they have been generally underestimated and studies are still required to tackle this issue (SAURA-CALIXTO, 2012). In addition, recent studies have shown that non-extractable polyphenols significantly contribute to the beneficial properties associated to dietary fibre (VITAGLIONE *et al*., 2008; SAURA-CALIXTO, 2011).

Table 3: TPC and FRAP values in aqueous-organic extracts and their residues in grains and flours*.

Products	Aqueous-organic extract (extractable polyphenols)					
	TPC (mg/100g d.m.)			FRAP (μmol/g d.m.)		
	Grain	Flour	P value$^{\Omega}$	Grain	Flour	P value$^{\Omega}$
SOLINA 1	170.43±28.61	129.28±13.64^{a}	0.01	6.60±0.47	2.75±0.17^{b}	0.0001
SOLINA 2	165.57±26.11	130.87±15.85^{a}	0.001	6.34±0.27	2.35±0.14^{a}	0.0001
SOLINA 3	183.75±14.07	155.24±12.74^{b}	0.01	6.47±0.60	2.31±0.07^{a}	0.0001
Mean	**173.25±24.05**	**138.46±18.16**	**0.0001**	**6.47±0.46**	**2.48±0.24**	**0.0001**

Products	Residue (hydrolysable polyphenols)					
	TPC (mg/100g d.m.)			FRAP (μmol/g d.m.)		
	Grain	Flour	P value$^{\Omega}$	Grain	Flour	P value$^{\Omega}$
SOLINA 1	1325.32±57.99^{c}	981.01±50.27^{b}	0.0001	109.12±10.54^{b}	114.25±3.24^{b}	n.s.
SOLINA 2	1084.42±66.99^{a}	811.65±38.57^{a}	0.0001	85.06±9.90^{a}	99.20±11.14^{a}	0.01
SOLINA 3	1198.73±37.49^{b}	791.50±80.14^{a}	0.0001	90.40±4.30^{a}	118.29±4.26^{b}	0.0001
Mean	**1202.82±114.39**	**866.82±104.77**	**0.0001**	**92.07±12.22**	**111.61±10.13**	**0.0001**

*mean ± s.d.; Anova, Tukey HSD Test: by column, means followed by different letters are significantly different ($p < 0.05$).

▫ Student's t test ; n.s.=not significant

In our study, the determined hydrolysable polyphenols (isolated in the residue of the aqueous-organic extract of grains) showed to account for 87-89% of total polyphenols and contribute to the total antioxidant properties for about 93%. These data match those obtained in the other studies on cereals (PEREZ-JIMENEZ and SAURA-CALIXTO, 2005; CHANRASEKARA and SHAHIDI, 2010).
Regarding the effect of milling process on TPC content, whole flours exhibited a more significant reduction than in grains, both in the aqueous-organic extract and in the residue. Results showed that antioxidant properties also appear to be affected by the milling process: a decrease in FRAP values was, in fact, observed in aqueous-organic extract (mean value: 6.47±0.46 *vs* 2.48±0.24 μmol/g d.m.; P<0.0001), whereas an increase in FRAP values was reported in residues (mean value: 92.07±12.22 *vs* 111.61±10.13 μmol/g d.m.; P<0.0001). The increase of FRAP values in residue after milling process could be due to a better efficiency of extraction of bound compounds with antioxidant properties, related to improvement of solvent penetration for the enlargement of the particle surface. These data confirm the importance of milling process in retention of components contributing to antioxidant properties (DUODU, 2011).
Nowadays, several investigations have been carried out to study the effects of cereal processing technologies, such as milling, baking, extrusion, etc., on antioxidant properties (LIYANA-PATHIRANA and SHAHIDI, 2007; RAGAEE *et al*., 2014). As a consequence, TPC (mg/100g d.m.) and FRAP (μmol/g d.m.) in aqueous-organic extracts and their residues were also studied in Solina bread samples, as reported in Table 4. It is worth mentioning that whole wheat bread represents a rich source of bioactive compounds as reported in literature (JENSEN *et al*., 2011; ABDEL-AAL and RABALSKI, 2013).

Table 4. TPC and FRAP values in aqueous-organic extracts and their residues in bread*.

Loaves	TPC (mg/100g d.m.)		FRAP (μmol/g d.m.)	
	Aqueous-organic extract	Residue	Aqueous-organic extract	Residue
Code A	63.13±6.03^{cd}	832.91±46.21^{a}	8.57±0.34^{d}	106.21±16.96^{a}
Code B	49.44±6.98^{b}	894.07±62.64^{a}	3.93±0.17^{a}	120.27±30.77^{ab}
Code C	52.87±6.93^{bc}	1326.90±48.00^{b}	6.14±0.34^{b}	141.90±8.33^{b}
Code D	36.96±5.59^{a}	1237.41±69.35^{b}	4.14±0.37^{a}	114.19±3.52^{a}
Code E	71.23±6.04^{d}	877.73±88.77^{a}	7.80±0.26^{c}	126.60±7.80^{ab}

*mean ± s.d.; Anova, Tukey HSD Test: by column, means followed by different letters are significantly different ($p < 0.05$).

In bread samples, as it was observed in raw materials, the major contribution to antioxidant properties was given by hydrolysable polyphenols (93-97%). Moreover, the comparison of the TPC and FRAP values of the residue in bread samples and the corresponding values in grains showed that a decrease of TPC, from grains to bread, corresponded to an increase of FRAP values. Several authors have observed that some antioxidants can be generated during non-enzymatic browning reactions, such as the Maillard reaction (MANZOCCO *et al*., 2001).

4. CONCLUSIONS

Our results showed that in grains and derivatives thereof, hydrolysable polyphenols (isolated in the residue of the aqueous-organic extract) represent an important fraction, with appreciable antioxidant properties and possibly health properties. In particular, this research highlighted that the analysis of potentially bioactive antioxidants, remaining in the residues, is required for an adequate and comprehensive determination of antioxidant capacity in cereals. Results also pointed out the key role played by the milling and baking process in antioxidant properties.
So, in conclusion, Solina wheat represents a valuable Italian traditional wheat cultivar with proved antioxidant properties. It also contributes to highlighting the benefits that traditional foodstuffs may have Europe-wide: they represent foods with an increased nutritional value and bring a strong contribution to land protection and conservation of biodiversity.

ACKNOWLEDGEMENTS

This work was undertaken within the project TERRAVITA, funded by the Italian Ministry of Agriculture, Food and Forestry Policies. The authors wish to thank Dr. D. Silveri, Dr. V. Galli and Mr. L. Bartoli for the technical assistance and Dr. Francesca Melini for the technical and linguistic assistance in the revision of this paper.

REFERENCES

Abdel-Aal E.M. and Rabalski I. 2013. Effect of baking on free and bound phenolic acids in wholegrain bakery products. Journal of Cereal Science 3:312.

Adom K.K., Sorrells M.E. and Liu R.H. 2003. Phytochemical profiles and antioxidant activity of wheat varieties. Journal of Agricultural and Food Chemistry 51: 7825.

American Association of Cereal Chemists. 2003. Approved Methods of the AACC, 10th edn. Methods 236 55-31, St. Paul, MN, The Association.

Arvola A., Lähteenmäki L., Dean M., Vassallo M., Winkelmann M., Claupein E., Saba A. and Shepherd R. 2007. Consumers' beliefs about whole and refined grain products in UK, Italy and Finland. Journal of Cereal Science 46: 197.

Aune D., Norat T., Romundstad P. and Vatten L.J. 2013. Whole grain and refined grain consumption and the risk of type 2 diabetes: a systematic review and dose-response meta-analysis of cohort studies. European Journal of Epidemiology 28: 845.

Benzie I.F.F. and Strain J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of ''antioxidant power'': the assay. Analytical Biochemistry 239: 70.

Carcea M., Durazzo A., Raguzzini A., Azzini E., Foddai M.S. and Maiani G. 2009. Bioactive components in wheat grains and their products. International Wheat Quality Conference-IV, Wheat science dynamics: challenges & opportunites, Saskatoon, 2-6 giugno 2009. Agrobios (International), 437-448.

Chandrasekara A. and Shahidi F. 2010. Content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. Journal of Agricultural and Food Chemistry 58: 6706.

Duodu K.G. 2011. Effects of Processing on Antioxidant Phenolics of Cereal and Legume Grains. In: Advances in Cereal Science: Implications to Food Processing and Health Promotion. Chapter 3, 31-54, 2011 American Chemical Society.

Durazzo A., Turfani V., Azzini E., Maiani G. and Carcea M. 2013. Phenols, lignans and antioxidant properties of legume and sweet chestnut flours. Food Chemistry 140: 666.

International Association for Cereal Science and Technology. 2003. Standard methods of ICC. Methods No. 104/1, 105/2, 110/1. Vienna, Austria: The Association.

Jensen S., Ostdal H., Skibsted L.H. and Thybo A.K. 2011. Antioxidants and shelf life of whole wheat bread. Journal of Cereal Science 53: 291.

Liyana-Pathirana C.M. and Shahidi F. 2007. The antioxidant potential of milling fractions from bread wheat and durum. Journal of Cereal Science 45: 238.

Liu R.H. 2007. Whole grain phytochemicals and health. Journal of Cereal Science 46: 207.

Manzocco L., Calligaris S., Mastrocola D., Nicoli M.C. and Lerici C.R. 2001. Review of non-enzymatic browning and antioxidant capacity in processed foods. Trends in Food Science and Technology 11: 340.

Mellen P., Walsh T. and Herrington D. 2008. Whole grain intake and cardiovascular disease. A meta-286 analysis. Nutrition Metabolism Cardiovascular Diseases 18: 283.

Okarter N. and Liu R.H. 2010. Health benefits of whole grain phytochemicals. Critical reviews in Food Science and Nutritional 50: 193.

Perez-Jimenez J. and Saura-Calixto F. 2005. Literature data may underestimate the actual antioxidant capacity of cereals. Journal of Agricultural and Food Chemistry 53: 5036.

Pérez-Jiménez J. and Torres J.L. 2011. Analysis of non-extractable phenolic compounds in foods: the current state of the art. Journal of Agricultural and Food Chemistry 59(24): 12713.

Pérez-Jiménez J., Diaz-Rubio M.E., Saura-Calixto F. 2013. Non-extractable polyphenols, a major dietary antioxidant: occurrence, metabolic fate and health effects. Nutrition Research Reviews 26: 118.

Pomeranz Y. and Williams P.C. 1990. Wheat hardness: Its genetic,structural, and biochemical background, measurement, and significance. Pages 471-548 in: Advances in Cereal Science and Technology, Vol. X. Y. Pomeran, Ed. AACC International: St. Paul, MN.

Porfiri O., Torricelli R., Silveri D.D., Papa R., Barbaccia G. and Negri V. 2001. The Triticeae genetic resources of central Italy: collection, evaluation and conservation. Hereditas 135: 187.

Pulido R., Bravo L. and Saura-Calixto F. 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. Journal of Agricultural and Food Chemistry 48: 3396.

Ragaee S., Seetharaman K. and Abdel-Aal el-SM. 2014. The impact of milling and thermal processing on phenolic compounds in cereal grains. Critical Reviews in Food Science and Nutrition 54: 837.

Schatzkin A., Mouw T., Park Y., Subar A.F., Kipnis V., Hollenbeck A., Leitzmann M.F. and Thompson F.E. 2007. Dietary fiber and whole-grain consumption in relation to colorectal cancer in the NIH-AARP Diet and Health Study. American Journal of Clinical Nutrition 85:1353.

Saura-Calixto F. 2011. Dietary fibre as a carrier of dietary antioxidants: an essential physiological function. Journal of Agricultural and Food Chemistry 59: 43-49.

Saura-Calixto F. 2012. Concept and health-related properties of nonextractable polyphenols: the missing dietary polyphenols. Journal of Agricultural and Food Chemistry 60: 11195.

Singleton V. L., Orthofer, R. and Lamuela-Raventos R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin- Ciocalteau reagent. Methods in Enzymology 299: 152.

Slavin J.L., Jacobs D. and Marquart L. 2000. Grain processing and nutrition. Critical Reviews in Food Science and Nutrition 40: 309.

Vitaglione P., Napolitano A. and Fogliano V. 2008. Cereal dietary fibre: a natural functional ingredient to deliver phenolic compounds into the gut. Trends Foods Science and Technology 19: 451.

Paper Received February 13, 2015 Accepted June 20, 2015