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Bioactive phytochemicals in wheat: Extraction, analysis, processing, and functional properties

Devanand L. Luthria *, *Yingjian Lu*, *K.M. Maria John*

U.S. Department of Agriculture, Food Composition Methods Development Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, Beltsville, MD 20705, USA

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ABSTRACT

Whole wheat provides a rich source of bioactive phytochemicals namely, phenolic acids, carotenoids, tocopherols, alkylresorcinols, benzoxazinoids, phytosterols, and lignans. This review provides information on the distribution, extractability, analysis, and functional properties of bioactive phytochemicals present in wheat. Understanding the impact of processing on wheat phytochemicals allows us to develop improved processes with higher retention of bioactive compounds in processed wheat foods. Details of extraction, analytical methodologies and processing effects on bioactive phytochemicals in wheat are presented in tabulated form.

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1. Introduction

In 2013, Food and Agriculture Organization (FAO) of the United Nations forecasted world cereal production will reach around 2500 million tonne and wheat production to be around 700 million tonne (FAO, 2014). Grains and its processed products are consumed globally as important energy sources. Grain-based foods provide the majority of the carbohydrates, some proteins, oils, dietary fiber, and other micronutrients in many diets. During the past few decades research publications from universities, governmental, non-profit health, industrial, and trade organizations have encouraged increased consumption of whole grain food products due to their positive health benefits (Chanson-Rolle et al., 2014; Shahidi & Chandrasekara, 2013). According to United States Food and Drug Administration (FDA), cereal grains that 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, should be considered a whole grain food (FDA, 2006). The germ contains the plant embryo and the endosperm contains starch and storage proteins. Bran is the outermost layer which protects the inner portion of the grain from external weather, insect molds, and other microorganisms attack.

Previous studies have indicated that consumption of whole grain foods can significantly reduce the risk of some chronic health conditions such as type 2 diabetes, cardiovascular disease, and cancer (Landberg, Marklund, Kamal-Eldin, & Åman, 2014; Seo et al., 2015; Tucker et al., 2014). Initially, the health beneficial effect of whole grains was primarily attributed to its high fiber content. However, recent research indicates that the beneficial effect of whole grain may arise from the combined action of several components such as fiber, vitamins, phenolics,

* *Corresponding author.* Research Chemist, Food Composition Methods Development Lab, Bldg. 161, Lab 202, BARC (E), 10300 Baltimore Avenue, Agricultural Research Service, US Department of Agriculture, Beltsville, MD 20705, USA. Tel: +1 301 504 7247; fax: +1 301 504 8314.
E-mail address: Dave.Luthria@ars.usda.gov (D.L. Luthria).
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carotenoids, alkylresorcinols, and other phytochemicals (Piironen, Lampi, Ekholm, Salmenkallio-Marttila, & Liukkonen, 2009). The major grains include wheat, rice, corn, oats, rye, barley, sorghum, triticale, millet, amaranth, and teff. In Asia, nearly half of the annually consumed grain is rice, while the major grain consumed in Europe and US is wheat. The bioactive phytochemicals in wheat can be broadly subdivided into the following categories: phenolic acids, carotenoids, tocopherols, alkylresorcinols, and other miscellaneous compounds (sterols, steryl ferulates, benzoxazinoids and lignans).

Phenolic acids recognized as simple phenols can be divided into two subgroups. One is hydroxybenzoic acid derivatives, which include vanillic, syringic, *p*-hydroxybenzoic, and gallic acids. The other is hydroxycinnamic acid derivatives, which include ferulic, *p*-coumaric, caffeic, and sinapic acids (Luthria & Liu, 2013; Verma, Hucl, & Chibbar, 2009). The concentration of phenolic acids in whole wheat ranges from approximately 200 to 1200 mg/g dry matter basis (DMB) (Andersson, Dimberg, Aman, & Landberg, 2014). Ferulic acid is the primary and most abundant phenolic acid in wheat grain. Smaller concentrations of *p*-hydroxybenzoic, vanillic, syringic, *o*-coumaric, *p*-coumaric, salicylic, sinapic acids are also present in wheat (Liyana-Pathirana & Shahidi, 2006; Moore et al., 2005).

Whole wheat grain also provides moderate sources of vitamin E. The vitamin E includes four tocopherols and tocotrienols. Tocopherols have saturated phytyl side chains, while tocotrienols have isoprenyl side chain with three double bonds. Previous studies have confirmed the presence of α-, β-, δ-, and γ-tocopherols in soft and hard wheat grain (Moore et al., 2005; Panfili, Alessandra, & Irano, 2003). The spelt, durum, and soft wheat grains from Italy contained vitamin E in a range from 56.5 to 74.3 μg/g dry weight, respectively, with 66–77% tocotrienols (Panfili et al., 2003). In addition, the concentration of total tocopherols and tocotrienols in whole wheat samples varied between 27.6 and 79.7 μg/g (Lampi, Nurmi, Ollilainen, & Piironen, 2008).

In whole wheat grains, color has been most commonly used as a quality indicator. The color is primarily attributed to the presence of carotenoids and their esters. The concentration of total carotenoids in whole wheat ranged from 0.8 to 2.17 μg/g (Moore et al., 2005). Lutein and zeaxanthin were the most predominant carotenoids present in whole wheat, with concentrations of 0.5–1.44 and 0.2–0.39 μg/g grain, respectively (Moore et al., 2005). In addition, lower concentrations of carotenoids including β-cryptoxanthin at 0.01–0.13 μg/g, and β-carotene at 0.09–0.21 μg/g have also been detected in wheat.

Phenolic lipids, also known as alkylresorcinols (ARs), are commonly present in wheat (Gunenc, Hadinezhad, Tamburic-Ilincic, Mayer, & Hosseninian, 2013). Structurally the ARs are similar to tocopherols except for the presence of a straight aliphatic hydrocarbon side chain and a single phenolic ring. The alkyl side chain may contain between 13 and 27 carbon atoms. 5-N-alkylresorcinols, 5-alkenylresorcinols, 5-oxoalkylresorcinols, 5-oxoalkenylresorcinols, and 5-hydroxyalkenylresorcinols are the five major classes of ARs reported in the whole wheat. ARs in whole wheat range from 489 to 1429 μg/g (Ross et al., 2003).

Other bioactive phytochemicals have also been extracted and identified in whole wheat. In this review, they are grouped together as a "miscellaneous" group. The miscellaneous group consists of benzoxazinoids, lignans, phytosterols and steryl ferulates. Benzoxazinoids are a group of potent natural compounds which have recently been identified in whole grain wheat and rye grains and in bakery products of these cereals (Hanhineva et al., 2011; Pedersen, Laursen, Mortensen, & Fomsgaard, 2011). The total benzoxazinoids content in wheat is very low (5 μg/g DMB). Phytosterols are isoprenoid compounds, which are biosynthetically derived from squalene. Phytosterols are mostly found in free or esterified forms, including esters of fatty acids and phenolic acids, as well as glycosides or acylated glycosides. According to the study of Nurmi, Nyström, Edelmann, Lampi, and Piironen (2008), the content of phytosterols range is 670–959 μg/g DMB in whole grain winter wheat and 797–949 μg/g DMB in spring wheat. A portion of the phytosterols occurs in ferulic acid ester form, i.e. as steryl ferulates.

This contribution provides an overview of bioactive phytochemicals such as phenolic acids, carotenoids, tocopherols, alkylresorcinols, and other miscellaneous bioactive compounds that are commonly found in wheat grain (Table 1). The distribution of bioactive phytochemicals, their extraction, analysis, changes during processing, varietal differences, and functional properties will be presented and discussed.

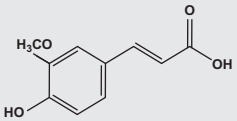
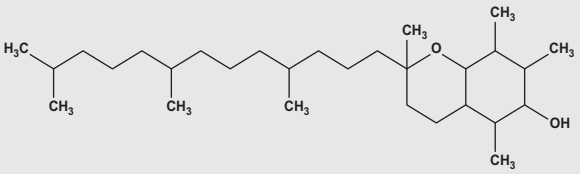
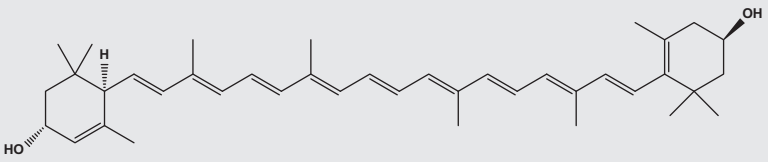
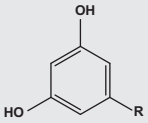
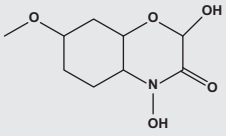
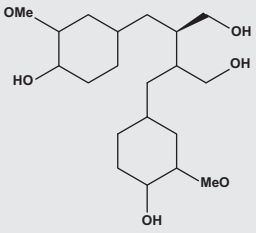
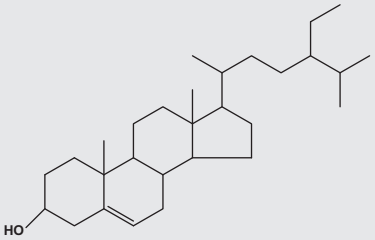
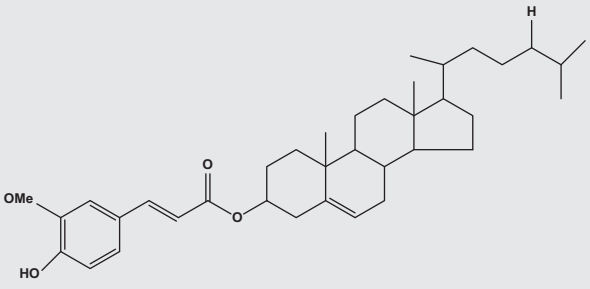
2. Distribution of bioactive phytochemicals in wheat

All whole wheat grain products contain bran, germ, and endosperm fractions. It is well documented in the published literature that the bioactive phytochemicals are not uniformly distributed. Germ and bran fractions generally contain higher concentration of bioactive phytochemicals. This is evident from number of pearling studies described in the literature (Blandino et al., 2013; Liyana-Pathirana, Dexter, & Shahidi, 2006). Liyana-Pathirana et al. (2006) studied the effect of sequential removal of the outermost layers (pearling) on the phenolic composition of wheat grains. Higher levels of phenolic compounds have been reported in the bran fraction as compared to the refined grain fraction that primarily comprises endosperm. In a recent study, it was reported that most of the phenolic acids existed in bound insoluble form (80%) as compared to free soluble form, and high concentrations of phenolic acids were detected in whole grain which contains the bran fraction (627.8–745.6 μg/g dry weight) as compared to the refined grain (66.0–97.0 μg/g dry weight) (Lu, Fuerst et al., 2014).

The concentration of total carotenoids in wheat ranges from 0.7 μg/g in durum wheat from Spain to as high as 13.6 μg/g in Einkorn accessions from Italy. The highest concentrations of carotenoids are observed in the germ fraction followed by the bran and endosperm fractions. Similarly, tocopherols and tocotrienols are not uniformly distributed in grain. Higher concentrations of tocopherols are found in the germ or the outer layers and the concentration of tocopherols in the endosperm fraction is comparatively lower. Similarly, tocotrienols are found at higher concentrations in outer layer of the grain (85%, pericarp, testa, and aleurone) and only 15% in the endosperm fraction (Piironen et al., 2009).

In a recent study, Tanwir, Fredholm, Gregersen, and Fomsgaard (2013) investigated the levels of benzoxazinoids in

Table 1 – Classification of bioactive phytochemicals present in wheat.

	Phytochemical name	Example	Structure
Phenolic acids	Hydroxy benzoic acid or hydroxy cinnamic acid derivatives	Ferulic acid	
Tocopherol	Tocopherols and tocotrienols	alpha-Tocopherol	
Carotenoids	Carotenoids	Lutein	
Phenolic lipids	Alkylresorcinols	5-alkenylresorcinols	
Miscellaneous groups	Benzoxazinoids	DIMBOA	
	Lignans	Secoisolariciresinol	
	Phytosterols	Sitosterol	
	Steryl ferulates	Campesteryl ferulate	

different wheat and rye fractions. Higher concentrations of benzoxazinoids were observed in the germ fraction than the bran and endosperm fractions. The fine bran was the second most enriched fraction. Phytosterols are known to accumulate in the bran and germ fractions of the wheat grain, whereas steryl ferulates are mostly found in the bran (Nurmi et al., 2012). The concentration of individual phytosterols, such as sitosterol, campesterol, sitostanol, campestanol, and stigmasterol, in wheat

was highly varied in their distribution (Nurmi et al., 2008). Lignans are polyphenolic substances derived from phenylalanine via dimerization of substituted cinnamic alcohols, known as monolignols, to a 2,3-dibenzylbutane structure. They are mostly concentrated in the bran fraction of the whole grain.

3. Extraction of phytochemicals in wheat

Optimized extraction of bioactive phytochemicals is of significant importance to accurately quantify the phytochemical content in foods and plant products. Accurate quantification allows establishment of proper dietary and safety guidelines. The bioactive phytochemicals exist in multiple forms: soluble (free and conjugated) and insoluble bound forms. It is essential to understand various forms of the bioactive phytochemicals to accurately quantify them and understand their bioavailability and health promoting properties. A wide array of extraction solvents and technologies have been reported in the peer-reviewed literature (Herrero, Cifuentes, & Ibañez, 2006; Luthria, 2012a). The conventional extraction methods include Soxhlet extraction, stirring, distillation, manual mixing, and percolation. However, in recent decades, several new technologies including, microwave assisted extraction, ultrasound-assisted extractions, pressurized liquid extraction, subcritical fluid extraction, solid-phase extraction, and enzyme-assisted extraction have been used to automate and/or improve the yields of bioactive phytochemicals (Table 2).

3.1. *Extraction of phenolic compounds*

The majority of wheat phenolic acids is present mainly in the bound form, linked to cell wall components such as cellulose, lignin, and proteins (Parker, Ng, & Waldron, 2005). The phenolic acids in wheat have three primary states: soluble free, soluble conjugated, and insoluble bound (Moore et al., 2005). The authors reported that most phenolic acids (~90%) occur in the insoluble bound and <9% and <1%, respectively, exist in soluble conjugated and soluble free forms. Alternatively, a simplified direct method in the presence of ascorbic acid and EDTA was used for the hydrolysis and extraction of total phenolic acids. This direct method provides marginal improvement in extraction of total phenolic acids from wide array of food matrices including wheat (Lu, Luthria et al., 2014).

Ultrasonic-assisted extraction (UAE) is an inexpensive and high-efficient extraction method. Simply, its mechanism depends on the collapse of bubbles that can accelerate the release of extractable compounds through the disruption of biological membranes and aids better solvent–solute interactions (Vinatoru, 2001). In the study of Hromadkova, Kostalova, and Ebringerova (2008), UAE was successfully used in the extraction of various phenolic compounds from wheat bran samples.

Microwave-assisted extraction (MAE) uses microwave energy to facilitate partition target compound from plant cells into the extraction solvent. The major advantage of MAE is the shortened extraction time with lower quantity of solvent as compared to conventional extraction method (Eskilsson & Bjorklund, 2000).

Pressurized liquid extraction (PLE) is a relatively new technology used for the extraction of phytochemicals under high temperature and pressure. With PLE, application of high pressure allows extraction above the normal boiling point of the extraction solvent. The combined use of high pressures (3.3–20.3 MPa) and high temperatures (40–200 °C) provides rapid extraction with reduced amounts of solvent (e.g., 20 min extraction with 10–50 mL of solvent using PLE provides similar or better extraction yields of phytochemicals as compared to traditional extraction method using 10–48 h and up to 200 mL of solvent) (Mendiola, Herrero, Cifuentes, & Ibanez, 2007). Buranov and Mazza (2009) extracted and purified the ferulic acid from wheat bran by alkaline hydrolysis using PLE. They indicated that crop residues contained pronounced levels of ferulic acid after extracting with PLE.

Solid-phase extraction (SPE) is widely used because it is a rapid, economical, and sensitive technique for partial purification of extracts. Different cartridges with varying numbers of sorbents can be used in SPE. Irakli, Samanidou, Biliaderis, and Papadoyannis (2012) developed and validated a high performance liquid chromatography (HPLC) method for determination of free and bound phenolic acids in cereals including wheat using solid-phase extraction on Oasis HLB cartridges with aqueous methanol as a solvent. These results showed that an optimized SPE method provided 95% recovery rate for phenolic acids from wheat (Arranz, Saura-Calixto, Shaha, & Kroon, 2009).

3.2. *Extraction of carotenoids and tocopherols*

Supercritical fluid extraction (SFE) has been applied to the extraction of compounds from wheat bran and germ as it offers several advantages namely, high selectivity and less extraction time and solvent cost (Herrero et al., 2006). Durante, Lenucci, Laddomada, Mita, and Caretto (2012) evaluated the effect of encapsulation on the stability of oil extracted by SFE-CO_2 from the durum wheat bran fraction. The authors showed that carotenoids were relatively stable at low temperature. However, an increase in temperature resulted in decreased tocopherol concentrations due to hydroperoxide formation caused by lipid peroxidation. They also indicated that encapsulation of SC-CO_2 extracted wheat bran oil in alginate beads resulted in a protective effect on nutritionally important compounds such as tocopherols, tocotrienols, lutein, zeaxanthin, and β-carotene. In addition, Gelmez, Kincal, and Yener (2009) investigated the influence of SFE on the measurement of the phenolic and tocopherol content of roasted wheat germ. Optimal extraction conditions for total phenolics and tocopherols were determined to be 336 bar (pressure), 58 °C (temperature), and 10 min (extraction time). The tocopherol yield under these conditions corresponded to almost 100% recovery.

Irakli, Samanidou, and Papadoyannis (2011) determined the carotenoids tocopherols and tocotrienols in seven cereals including bread wheat and durum wheat by HPLC. The extraction method included sample saponification and clean-up by SPE using OASIS HLB SPE cartridges. The recoveries for each tocopherol and carotenoid compound ranged from 90.2 to 110.1% with RSD lower than 10%. In a separate research, the same group optimized a RP-HPLC method using a novel sorbent material (PerfectSil Target ODS-3 column) for the separation of tocopherols and tocotrienols in durum wheat and bread wheat. The SPE method was applied to extract the vitamin E components.

Table 2 – Methods used for the extraction of bioactive phytochemicals from wheat and its processed products.

Serial no.	Extraction	Phytochemicals	Results	Changes in levels of phenolics [a]	References
1	Conventional solvent extraction	TPC	Different solvents showed different efficacies for extraction of phenolic compounds. The extract produced with 60% v/v acetone had the highest content of total phenols.	+	Meneses, Martins, Teixeira, & Mussatto, 2013
2	Ultrasonic-assisted extraction (UAE)	Phenolics-rich heteroxylans	UAE represents a shortening of the process by about 60% and lower consumption of the NaOH.	+	Hromadkova, Kostalova, & Ebringerova, 2008
3	Microwave-assisted extraction (MAE)	TPC	There was little difference of levels of phenolic acids between heating with microwave irradiation and the same conditions using a regular water bath as the heat source.	*	Inglett, Rose, Stevenson, Chen, & Biswas, 2009
4	Pressurized liquid extraction (PLE)	Phenolic acid	The production of vanillin from crop residues containing greater levels of ferulic acid such as corn bran and sugar-beet pulp with PLE may be practical and cost-effective.	+	Buranov & Mazza, 2009
5	Enzymatic extraction	Polyphenols	Enzymatic extraction seems to be more efficient than organic solvents for phenol bioaccessibility in wheat bread.	+	Angioloni & Collar, 2012
6	Solid-phase extraction (SPE)	Phenolic acid	Using an optimized solid-phase extraction (SPE) method a range of 84% to 106% recovery rate was obtained for phenolic acids in cereals including wheat.	+	Irakli, Samanidou, Biliaderis, & Papadoyannis, 2012
7	Supercritical fluid extraction (SFE)	Tocopherols	Pressure of 4000–5000 psi, the extracting temperature of 40–45 °C, and the carbon dioxide flow rate of 2.0 mL/min for 90 min were their optimal extraction conditions to extract tocopherols from wheat germ.	*	Ge, Yan et al., 2002
8	SFE	Tocopherols and tocotrienols	Optimum value predicted by RSM for the concentration of natural vitamin E was 2307 mg/100g.	*	Ge, Ni, Yan, Chen, & Cai, 2002
9	SFE	Tocopherols and tocotrienols	Tocopherol yield was 0.33 mg tocopherol/g germ at 405 bar, 60 °C and 10 min).	*	Gelmez, Kincal, & Yener, 2009
10	SFE	Tocopherols, tocotrienols, and carotenoids	Encapsulation of SC-CO_2 extracted wheat bran oil in alginate beads resulted in a protective effect on nutritionally important compounds such as tocopherols, tocotrienols, lutein, zeaxanthin, and β-carotene.	+	Durante, Lenucci, Laddomada, Mita, & Caretto, 2012
11	Conventional solvent extraction	Carotenoids and tocopherols	Butanol method extraction is best for carotenoid extraction, while hot saponification and the butanol protocols have a better extraction efficiency than the other extraction method for tocopherols.	+	Hidalgo, Brandolini, Pompei, & Piscozzi, 2006
12	Conventional solvent extraction	Alkylresorcinols	Acetone used as extraction solvent in a 1:40 (w/v) ratio followed by continuous stirring (Stirrer-VWR, Corning®, VMS-C4) at room temperature for 24h	*	Gunenc, Hadinezhad, Tamburic-Ilincic, Mayer, & Hosseninian, 2013
13	Conventional solvent extraction	Alkylresorcinols	Ethyl acetate used as solvent at room temperature under periodic hand shaking for 48 h. After extraction the solvent layer was filtered and evaporated to dryness before analysis	*	Sampietro et al., 2013
14	Conventional solvent extraction	Alkylresorcinols	Propanol:water (3:1) for the extraction of ARs from the processed food was reported	*	Holt, Moreau, DerMarderosian, McKeown, & Jacques, 2012
15	SFE	Alkylresorcinols	CO_2 desired operating pressure: 40.0 MPa, extracting temperature of 40–80 °C, and the carbon dioxide flow rate of 1.5 ± 0.3 g/min was used for the extraction and the results were compared with conventional solvent extraction. The extraction yield was higher for polar organic solvents than for SC-CO_2	–	Rebolleda, Beltrán, Sanz, González-Sanjosé, & Solaesa, 2013
	Conventional solvent extraction and SFE	Alkylresorcinols	No qualitative or quantitative variations between the two extraction methods	*	Landberg, Dey, Francisco, Aman, & Kamal-Eldin, 2007

(continued on next page)

Table 2 – (*continued*)

Serial no.	Extraction	Phytochemicals	Results	Changes in levels of phenolics	References
16	Ultrasonic-assisted extraction (UAE)	Alkylresorcinols	Dichloromethane was used under ultra-sound-assisted extraction method and the samples were sonicated for 15 s at 50% amplitude. During ultrasonication, samples were cooled in an ice bath to avoid sample heating. The duration of analytical extraction was shortened from more than 1 h to only 45 s as compared to previous methods. In addition, sample weight and solvent use were significantly reduced.	+	Christian, Anna, Reinhold, & Ralf, 2015
17	Accelerated solvent extraction (ASE)	Benzoxazinoids	Dionex ASE 350 Accelerated Solvent Extractor was used with the extraction solvent containing 19% water, 80% methanol and 1% acetic acid. The extraction was carried out at 80 °C.	*	Pedersen, Laursen, Mortensen, & Fomsgaard, 2011
18	Acid/base hydrolyses	Phytosterols	The phytosterols were extracted by using acid and base hydrolyses of the samples followed by silica gel column purification prior to the analysis	*	Nurmi, Nyström, Edelmann, Lampi, & Piironen, 2008
19	ASE	Phytosterols	Different extraction solvents with different temperature were used to optimize the extraction condition using ASE.	*	Dunford, Irmak, Jonnala, 2009
20	Hot acetone under reflux	Steryl ferulates	Hot acetone under reflux followed by base–acid for the purification of the extract	*	Nurmi et al., 2012
21	Alkaline hydrolysis/ enzyme digest	Lignans	The alkaline hydrolyzed samples were column purified using SPE column	*	Cukelj, Jakasa, Sarajlija, Novotni, & Curic, 2011

[a](+) increase, (–) decrease or (*) no change in levels of plant secondary chemical.

They found that SPE provided a high extraction recovery for durum wheat and bread wheat: 87.0% for γ-tocotrienol and 100% for δ-tocopherol (Irakli, Samanidou, & Papadoyannis, 2012).

Rezaee et al. (2006) used dispersive liquid–liquid microextraction (DLLME) for the extraction of tocopherols and tocotrienols. Shammugasamy, Ramakrishnan, Ghazali, and Muhammad (2013) determined tocopherols and tocotrienols in cereals including wheat by reverse phase HPLC after extraction by combination of saponification and DLLME. The authors concluded that DLLME method is precise and accurate in determining the tocopherol and tocotrienol contents in cereals. The advantages of this method were reduced extraction time and comparatively less solvent usage therefore cost efficient.

Hidalgo, Brandolini, Pompei, and Piscozzi (2006) used three different solvents for extraction of carotenoids (water-saturated 1-butanol, hot saponification, and tetrahydrofuran) from einkorn wheat samples. The authors concluded that butanol method was better for carotenoids extraction, while hot saponification and the butanol protocols had better extraction efficiency for tocopherols.

3.3. *Extraction of alkylresorcinols (ARs)*

Various solvents, such as acetone, ethyl acetate, dichloromethane, and cyclohexane have been reported in literature for the extraction of ARs. The conventional extraction method utilizes refluxing sample with extraction solvent for period ranging from 1 to 48 h. The extracts are filtered, evaporated, dried and reconstituted in a suitable solvent prior to analysis (Zarnowski & Suzuki, 2004). Recently, a direct comparison of supercritical CO_2 and conventional ethyl acetate extraction was carried out by Landberg, Dey, Francisco, Aman, and Kamal-Eldin (2007), Gunenc, HadiNezhad, Farah, Hashem, and Hosseinian (2015). The authors reported that no qualitative or quantitative variations between the two extraction methods were detected. Christian, Anna, Reinhold, and Ralf (2015) reported ultrasound-assisted extraction with dichloromethane. The interesting aspect of the Christian's method was direct extraction of ARs from the outer bran fraction of the grain without grinding. Similar extraction was carried out by Zarnowski and Suzuki (2004) by refluxing the whole grain with the selected solvents.

3.4. *Extraction of miscellaneous bioactive phytochemicals*

The miscellaneous group of phytochemicals includes benzoxazinoids, sterols, lignans, and steryl ferulates. Extraction of benzoxazinoids requires hydrolysis of the glycosides to aglycons followed by enzymatic treatment to obtain stable benzoxazolinones. The benzoxazinoids were extracted by soaking the wheat samples in water for a period of 6 h at room temperature followed by boiling with water for an additional 15 min. The water extracts were then filtered, lyophilized, and powdered. The lyophilized powder was extracted by 80% methanol, 1% acetic acid, and 19% water (v/v) in a Dionex ASE 350 accelerated solvent extractor. The benzoxazinoids were extracted using the following conditions: temperature, 80 °C; heat, 5 min; static time, 3 min; cycles, 4; rinse volume, 60%; purge, 60 s (Pedersen et al., 2011; Tanwir et al., 2013).

Phytosterols were extracted by acid and/or base hydrolysis of the wheat sample followed by silica column purification.

However, steryl ferulates and other phenolic acid esters were extracted using hot acetone under reflux followed by a base–acid wash (Nurmi et al., 2012). The extraction method for lignans used an initial alkaline hydrolysis of the wheat bran followed by incubation with an enzyme (pomatia-glucuronidase/sulfatase). After incubation the mixture was purified on a SPE column. The ethanol:water solvent mixture was used for the lignan extraction. The extracts were acid hydrolyzed and filtered before analysis (Zhang et al., 2007).

4. Analysis of phytochemicals in wheat

The analytical methods used for the determination of phytochemicals in whole grains are continuously changing due to rapid advances in technology. The method used for analysis depends on the resources available and the research goals (Table 3). High performance liquid chromatography (HPLC) and gas chromatography (GC) methods have been extensively used for separation, identification, and quantification of phytochemical compounds in wheat samples (Gunenc et al., 2013; Lu, Lv et al., 2014; Luthria, 2012b). HPLC with diode array detection (DAD) is the most popular and reliable technique and is commonly preferred over GC which frequently requires an additional derivatization step. Spectroscopic techniques including mass spectrometry (MS), nuclear magnetic resonance (NMR), and near-infrared (NIR) detections are commonly used for structural elucidation, qualitative, and quantitative analysis (Nicoletti et al., 2013; Sun, Sun, Fowler, & Baird, 2005).

4.1. *Analysis of phenolic acids*

HPLC-DAD and HPLC-MS are frequently used for the analysis of phenolic acids in wheat. In previous studies, Lu, Lv et al. (2014) determined the insoluble and soluble ferulic acid content in ten soft wheat bran samples using HPLC. The authors indicated that around 99% of the ferulic acid was present in an insoluble bound form in wheat bran samples. In addition, Nicoletti et al. (2013) investigated the soluble free, conjugated, and insoluble bound phenolic acids in durum wheat using LC-MS. Their results showed that the total content of the three different forms of phenolic acids was in the order *insoluble bound > soluble conjugated > soluble free*. The authors also pointed out that ferulic acid was the predominant phenolic acid in the soluble free and insoluble bound forms, whereas sinapic acid was the predominant phenolic acid in the soluble conjugated form. Dinelli et al. (2009) analyzed various phenolic compounds including phenolic acids, flavonoids, coumarins, proanthocyanidins, stilbenes, and lignans from old and modern varieties of durum wheat using HPLC coupled with time-of-flight (TOF)-MS. They concluded that old wheat varieties may provide unique functional properties for their bioactive phytochemical content, suggesting their use into a broad range of regular and special products. NMR spectroscopy is used for structural elucidation of bioactive phytochemicals. It has also been used recently for metabolomic studies. Sun et al. (2005) identified and characterized *p*-hydroxybenzoic acid, vanillic acid, syringic acid, *p*-coumaric acid, ferulic acid, and cinnamic acid, and lignin in wheat straw by using NMR and NIR. In addition, minor quantities of esterified or etherified *p*-coumaric (0.06%) and ferulic acid (0.08%) were observed in the lignin preparations, suggesting that these two hydroxycinnamic acids are strongly linked to lignins.

4.2. *Analysis of carotenoids and tocopherols*

Tocopherols and carotenoids are also commonly analyzed by HPLC-MS and GC–MS. Hung and Hatcher (2011) developed a fast and sensitive method for separation of carotenoids from durum wheat flours by uHPLC. The peaks of all carotenoid components were detected at 445 nm using a photodiode array detector. Their results showed that two wheat varieties (Commander and AC Navigator) contained higher lutein concentration (5.31 and 5.59 μg/g flour, respectively) than the other cultivars, and wheat grown in the Taber and Swift Current regions had greater lutein concentration (ranging from 0.45 to 5.59 μg/g flour) than those grown in Regina (ranging from 0.37 to 3.98 μg/g flour). Similarly, Ndolo and Beta (2013) investigated the distribution of the carotenoids in endosperm, germ, and aleurone fractions of four soft wheat cultivars using HPLC. The results indicated that the germ fraction of wheat had significant levels of lutein (2157 μg/kg) and zeaxanthin (3094 μg/kg). Lower amounts of lutein (557 μg/kg) and zeaxanthin (not detected) were found in the wheat endosperm. In addition, the average zeaxanthin content was 3.5 times higher in wheat germ than in yellow corn.

Beleggia, Platani, Nigrp, De Vita, and Cattivelli (2013) examined the metabolite profiles of durum wheat using GC and GC–MS. The authors identified tocopherols, phytosterols, amino acids as well as other metabolites using GC–MS. This method can provide a basis for developing improved wheat cultivars with increased nutritional value and health benefits for durum wheat. Lu, Lv et al. (2014) investigated the levels of carotenoids and tocopherols in ten soft wheat bran samples using HPLC. Their results showed that α-tocopherol (2.3–5.3 μg/g) and lutein (1.0–1.4 μg/g) were the predominant tocopherols and carotenoids in all wheat bran samples. In another study, Giambaneli et al. (2013) analyzed the tocopherol and carotenoid contents in primitive wheat populations from European countries by HPLC. The authors showed that wheat genotypes affected the relative levels of α-tocopherol, β-tocopherol, α-tocotrienol, and β-tocotrienol. Lutein was the most predominant catortenoid in all the samples. The total carotenoids contents in *Triticum monococcum*, *Triticum timopheevi* and *Triticum durum* ranged from 0.567 to 0.785 mg/mg. *Triticum dicoccum* contained the lowest relative lutein content, but the highest zeaxanthin concentration (0.272 mg/mg total carotenoids).

Similar studies regarding the carotenoid and tocopherol determinations in wheat or wheat-based products were also reported (Lv et al., 2013; Moore, Luther, Cheng, & Yu, 2009). In a recent study, Kumar and Krishna (2013) used HPLC and NMR (^{13}C and ^{1}H) to determine the distribution of tocopherols and carotenoids in the unsaponifiable matter of wheat bran and wheat germ oils. These results showed that wheat germ oil contained higher concentrations of tocopherols and carotenoids (273 and 12.23 mg/100 g, respectively) than wheat bran oil (190 and 2.21 mg/100 g, respectively). The total carotenoids could also be determined as a measure of total xanthophylls in wheat flour and wheat grits by a spectrophotometric method (Luterotti

Table 3 – Separation, identification, and metabolic profiling of bioactive phytochemicals in wheat and wheat-based products.

Serial no.	Analysis	Phytochemicals	Results	References
1	HPLC	Ferulic acid	Around 99% of the ferulic acid was identified as insoluble bound form in wheat bran samples	Lu, Lv et al., 2014
2	LC-MS	Phenolic acids	Total content of the three different forms of phenolic acid is in the order bound > conjugated > free in each of durum wheat sample.	Nicoletti et al., 2013
3	LC-MS	Phenolic acids, flavonoids, coumarins, proanthocyanidins, stilbenes, and lignans	Old wheat varieties may provide unique functional properties for their peculiar contents in bioactive phytochemicals.	Dinelli et al., 2009
4	NMR	Phenolic acids and lignin	Phenolic acids and lignin in wheat straw by using NMR	Sun, Sun, Fowler, Baird, 2005
5	NIR	Phenolic acids and lignin	Phenolic acids and lignin in wheat straw by using NIR	Sun, Sun, Fowler, Baird, 2005
6	GC, GC–MS	Tocopherols and phytosterols	GC and GC–MS help to provide a basis for developing improved wheat cultivars with increased nutritional value and health benefits for durum wheat	Beleggia, Platani, Nigrp, De Vita, and Cattivelli, 2013
7	HPLC	Carotenoids and tocopherols	α-Tocopherol and lutein is the predominant tocopherol and carotenoid in all wheat bran samples.	Lu, Lv et al., 2014
8	HPLC	Carotenoids and tocopherols	Wheat varieties affected the relative levels of all the tocopherol components and carotenoids whereas sowing data had slight effect.	Giambaneli et al., 2013
9	HPLC and NMR	Tocopherols and carotenoids	Wheat germ oil contained higher concentrations of tocopherols and carotenoids (273 mg/100 g, 12.23 mg/100 g) than wheat bran oil (190 mg/100 g, 2.21 mg/100 g), respectively.	Kumar & Krishna, 2013
10	Colorimetric method	Alkylresorcinols	ARs was coupled with Fast Blue RR salt in alkaline medium, yielding colored azo-derivatives and were quantified by colorimeter. Good linearity was observed between the samples	Gajda Kulawinek, & Kozubek , 2008; Sampietro, Vattuone & Catalán, 2009
11	HPLC	Alkylresorcinols	The ARS were analyzed by using HPLC containing Kromasil C18-5 column. Methanol (A) and water (B) served as mobile phase	Rebolleda, Beltrán, Sanz, González-Sanjosé, & Solaesa 2013
12	GC–MS	Alkylresorcinols	The extracted metabolites were silylated with 0.5 mL pyridine:HMDS:TMCS (9:3:1)	Landberg, Dey, Francisco, Aman, & Kamal-Eldin, 2007
13	GC and NMR	Alkylresorcinols	ARs were analyzed by GC and the characterization was made by using NMR.	Zarnowski & Suzuki, 2004
14	HPLC/MS	Benzoxazinoids	UPLC BEH C18 column, 0.1%formic acid in acetonitrile/ water (5:95, v/v) (phase A) and 0.1% formic acid in acetonitrile (phase B). ESI conditions are capillary voltage at 3.0 kV; cone voltage at 30 eV; collision energy at 3 eV and 20 eV; argon used as collision gas.	Hanhineva et al., 2011
15	GC-FID	Phytosterols	TMS derivatized samples were analyzed by GC-FID equipped with a 5% diphenyl–95% dimethyl polysiloxane column	Nurmi et al., 2008
16	HPLC/-MS	Steryl ferulates	The methanol/water (98:2) served as mobile phase. The MS analysis performed with APCI at the interface temperature at 400 °C, nebulizer gas pressure at 50 psi, voltage of the corona discharge needle was –1.5 kV.	Hakala et al., 2002
17	GC–MS	Lignans	Pyridine and pentafluoropropionic anhydride (PFPA) were used for the derivatization of the metabolites	Cukelj, Jakasa, Sarajlija, Novotni, & Curic, 2011

& Kljak, 2010). The authors indicated that the total carotenoids levels in the wheat flours was 1.1–1.3 mg/kg, and in wheat grits 1.6 mg/kg.

4.3. Analysis of alkylresorcinols (ARs)

ARs are commonly analyzed by three different techniques, colorimetric, GC–MS, and HPLC-MS. The colorimetric method of detection and quantification is simple, reliable, and inexpensive. The colorimetric method based on diazonium salt Fast Blue B BF_4 developed by Tluscik, Kozubek, and Mejbaum-Katzenellenbogen (1981) has been modified several times. Initially the modification was due to the unavailability of the Fast Blue BF_4 reagent that was replaced by Fast Blue B $ZnCl_2$ salt that was commercially available. Later chemical modifications were driven by optimization of sample throughput and stability of the colored complex. Recently, Sampietro, Jimenez, Belizán, Vattuone, and Catalán (2013) developed a micro method for the quantification of 5-n-alkylresorcinols in grain and grain products. In this method 5-N alkylresorcinols are detected from ground and intact whole grains using Fast Blue RR ½ $ZnCl_2$ to yield colored azo derivatives that were measured at 490 nm with a shorter incubation time (15 min). This rapid method correlated well ($R^2 = 0.9944$) with the commonly used Fast Blue B method and absorbance measurement of colored azo complex at 520 nm.

Chromatographic separation using HPLC and GC with UV, fluorescence (FD), Coulombic array electrochemical detection (CAED), and MS detection methods has been used for the analysis of alkylresorcinols. Some HPLC methods have longer run times due to poor resolution between the unsaturated homologs and saturated side chains. However, GC methods provide comparatively better resolution and are commonly used for the analysis of alkylresorcinols. Advances in HPLC that allow higher back pressure (>1000 bar) and smaller particle size columns (<3 μm) provide better resolution of ARs. Ross et al. (2012) developed a HPLC method for the separation of alkylresorcinols from 15 cereal grains and 90 cereal products and compared the performance of three different detectors (UV, FD, and CAED). The limit of quantification and detection for the three methods were significantly different. CAED was the most sensitive followed by FD and UV. The authors also pointed out that UV detection generally resulted in an over-estimation of alkylresorcinols concentration.

Gas chromatography with MS and flame ionization detections (FID) has been used for the analysis of alkylresorcinols. In most procedures, silyl derivatives of alkylresorcinols are prepared to improve the volatility of the compounds. Landberg, Aman, and Kamal Eldin (2009) used an OasisMax solid phase extraction cartridge for sample cleanup to develop a rapid GC–MS method for the quantification of alkyl resorcinols in human plasma.

4.4. Analysis of miscellaneous bioactive phytochemicals

Dinelli et al. (2009) reported phytochemical profiles of wheat by HPLC-ESI-TOF-MS. In this experiment, a RP C18 analytical column with water and acetonitrile acidified with 0.5% acetic acid (v/v, mobile A and B) was used for the separation of the phytochemicals. The ESI-MS parameters (capillary voltage, +4.5 kV; drying gas temperature, 190 °C; drying gas flow, 7.0 L/min; and nebulizing gas pressure, 21.7 psi with the mass range from m/z 50 to 1000) were optimized for the profiling of 70 phytochemicals including flavonoids, phenolic acids, and proanthocyanidins in wheat.

Phytosterols and steryl ferulates were analyzed using GC and HPLC techniques. For the phytosterol analysis, the extracts were derivatized before injecting into the GC system (Nurmi et al., 2008). The individual phytosterols, sitosterol, campesterol, sitostanol, campestanol and stigmasterol, were reported. HPLC (Nurmi et al., 2012) and HPLC-MS (Hakala et al., 2002) based analysis of steryl ferulates indicated that the levels of individual steryl ferulates (sitosteryl ferulate, sitostanyl ferulate, and campesteryl ferulate) in wheat were highly varied in their distribution. Lignans were analyzed by Cukelj, Jakasa, Sarajlija, Novotni, and Curic (2011) using GC–MS after derivatizing the extracts with pentafluoropropionic anhydride (PFPA) to improve the volatility of the metabolites.

5. Phytochemicals in grains during processing

Wheat is commonly processed prior to consumption. Grain producers, processors, consumers, health, and nutritional professionals are interested in investigating the effect of processing on bioactive phytochemicals present in wheat (Wang, He, & Chen, 2014). The stability of bioactive phytochemicals is influenced by processes (milling, fermentative proofing, baking, enzymatic reaction, extrusion, cooking, steaming, malting, etc.) and its conditions (temperature, light, pressure, time, etc.). This information is critical to the development of optimal processes capable of preserving bioactive phytochemicals in wheat based finished products. In this review, we briefly summarize the effect of processing on bioactive phytochemicals namely, phenolic acids, carotenoids, tocopherols, alkyl resorcinols, arabionxylans and other bioactive phytochemicals (Table 4).

5.1. Phenolic compounds during processing

Phenolic acids are the predominant phytochemicals present in grain. Ultrafine grinding used by Rosa, Barron, Gaiani, Dufour, and Micard (2013) increased the bioaccessibility of phenolic acids from wheat bran. The authors reported that wheat bran fractions had 1.5-fold increased antioxidant capacity when the particle size decreased from 172 to 30 μm using ultra-fine grinding. The antioxidant activity was inversely related to the particle size of the wheat bran fraction. In agreement to the above study, Hemery et al. (2010) examined the potential of using ultrafine grinding of wheat bran as a method to improve the bioaccessibility of *p*-coumaric acid, sinapic acid, and ferulic acid. The authors indicated that ultra-fine grinding increased the particle surface area of wheat bran, thus finally leading to the release of more *p*-coumaric acid, sinapic acid, and ferulic acid. In another recent study, Brewer, Kubola, Siriamornpun, Herald, and Shi (2014) showed that the fine milling treatment resulted in higher levels of phenolic acids, flavonoids, anthocyanin, and carotenoids as compared to unmilled treatment.

Table 4 – The effect of processing on the level of phytochemicals in wheat and its products.

Serial no.	Processing	Phytochemicals	Results	Changes in levels of phenolics [a]	References
1	Ultra-fine grinding	Phenolic acids	Wheat bran fractions had 1.5-fold increased antioxidant capacity when the particle size decreased from 172 to 30 μm using ultra-fine grinding.	+	Rosa, Barron, Gaiani, Dufour, & Micard, 2013
2	Ultra-fine grinding	Phenolic acids	Ultra-fine grinding increased the particle surface area of wheat bran, thus finally leading to release of more p-coumaric acid and ferulic acid.	+	Hemery et al., 2010
3	Milling	Phenolic acids, flavonoid, anthocyanin, and carotenoid	Significant increase in extracted anthocyanins, carotenoids, and ORAC value was observed, and particle size does influence the extraction of phytochemicals in wheat bran.	+	Brewer, Kubola, Siriamornpun, Herald, & Shi, 2014
4	Fermentation	Total phenolics	The fermented wheat grains prepared with two filamentous fungi had higher levels of phenolics as compared to non-fermented wheat grains koji.	+	Bhanja, Kumari, & Banerjee, 2009
5	Baking	Polyphenols and flavonoids	Polyphenol content was found to be decreased in the bread samples, whereas quercetin content increased significantly in buckwheat with bread-making. The baking decreased the levels of flavonoids.	–	Alvarez-Jubete, Wijingaard, Arendt, & Gallagher, 2010
6	Baking	Phenolic acids	Baking resulted in an increase in free phenolic acids in the three products, while bound phenolic acids decreased in bread and slightly changed in cookie and muffin products.	+/–	Abdel-Aal & Rabalski, 2013
7	Baking	Phenolic acids, tocopherols, and carotenoid	Baking reduced the concentrations of carotenoids and tocopherols, however, total phenolic acids content might not decrease during baking.	*/–	Lu, Fuerst et al., 2014
8	Baking	Phenolic acids	The total quantified phenolic acids did not change significantly, when preparing bread from refined and whole wheat flour.	*	Lu, Luthria et al., 2014
10	Toasting	Total phenolics	Longer toasting of defatted soy flakes at 150 °C resulted in increasing aglycone levels and total phenolics.	+	Pananun et al., 2012
11	Heating	Carotenoids/ Tocopherols	Dry heat treatment increased the vitamin E and reduced the carotenoids in sorghum. The wet heat reduced all antioxidant compounds except carotenoids, which increased.	+/–	Cardoso et al., 2014
12	Heating	Carotenoids and total tocopherols	Fat-soluble compounds total tocopherols, steryl ferulates and carotenoids found in wheat germ started to get the maximum reductions (0.183%, 0.034% and 0.004%) at 130 °C.	–	Kumar, Swathi, & Krishna, 2014
13	Baking	Carotenoids	Baking of β-carotene fortified bread and cracker decreased total carotenoids from 4 to 23%.	–	Ranhotra, Gelroth, Langemeier, & Rodgers 1995
14	Baking	Carotenoids	Baking resulted in 36%–45% losses of carotenoids in wheat-based products.	–	Leehardt et al., 2006
15	Fermentation	Carotenoids	10% of losses in total carotenoids as a result of dough fermentation at 30 °C.	–	Leehardt et al., 2006
16	Sprouting	Polyphenols	Sprouting of wheat appeared to significantly increase its polyphenol content.	+	Alvarez-Jubete, Wijingaard, Arendt, & Gallagher, 2010
17	Kneading	Carotenoids	66% decrease in total carotenoid contents in wheat samples during kneading was observed.	–	Leehardt et al., 2006
18	Mixing	Tocopherols	The content of tocopherol decreased during mixing for all products (21.4%, 28.2%, and 44.2% for bread, biscuit and pasta, respectively)	–	Hidalgo & Brandolini, 2010
19	Baking	Alkylresorcinols	To denaturation or degradation of ARs during processing was reported.	–	Weipert & Al Baya 1977; Winata & Lorenz, 1997
20	Wet processing	Alkylresorcinols	Bound with flour and starch–lipid complexes formation	*	Morrison Milligan, & Azudin, 1984
21	Hydrothermal processing (HTP) and baking	Benzoxazinoids	Enzymatic liberation of aglycones and their subsequent degradation to benzoxazolinone	–	Pedersen, Laursen, Mortensen, & Fomsgaard, 2011
22	Frying	Phytosterols	Due to the oxidation process of phytosterols, their levels reduced in the processed food	–	Soupas, Huikko, Lampi, & Piironen, 2007
23	Roasting/wet processing	Lignan	Decrease the lignan content due to enzymatic activity	–	Gerstenmeyer, Reimer,Berghofer, Schwartz, & Sontag, 2013

[a](+) increase, (–) decrease or (*) no change in levels of plant secondary chemical.

Fermentation processes are commonly used in the food industry. Bacteria and yeasts are used to convert carbohydrates into alcohols, CO_2, or carboxylic acids. Many metabolizing enzymes can be generated during fermentation. These enzymes can further hydrolyze the phenolic glycosides into free phenolic acids. For instance, Bhanja, Kumari, and Banerjee (2009) found that fermented wheat grains prepared with two filamentous fungi had higher levels of phenolics as compared to non-fermented wheat grains koji.

Different thermal processing methods such as baking, cooking, toasting, and microwaving have varying effects on the phenolic compounds in whole wheat. Abdel-Aal and Rabalski (2013) studied the effect of baking on free and bound phenolic acids in wheat bakery products. They found that ferulic acid was the predominant phenolic acid both in the free or bound form of three whole wheat bakery products (bread, cookie, and muffin). Most importantly, their results showed that baking increased the free phenolic acids in the three bakery products, while bound phenolic acids decreased in bread and were only slightly changed in cookie and muffin products. Polyphenol content was generally found to be decreased in the bread samples as compared to their raw grain. Recently, Lu, Luthria et al. (2014) investigated the influence of baking on the total phenolic acids in dough (mixed and proofed) and three bread fractions (upper crust, crumbs, and bottom crust) made from refined and whole-wheat flour of three wheat varieties. The results showed that the total quantified phenolic acids did not change significantly when preparing bread from refined and whole wheat flour. The concentration of the total phenolic acids in all whole wheat bread fractions were 5.5- to 9.8-fold greater than the corresponding refined wheat fractions. In addition, the results indicated that the upper crust fraction had higher levels of total phenolic acids than the dough and crumb fractions, suggesting that total phenolic acids content did not decrease significantly during baking (Lu, Fuerst et al., 2014; Lu, Luthria et al., 2014).

5.2. *Carotenoids and tocopherols during processing*

Carotenoids are known to be sensitive to light and heat (Mercadante, 2007). Ranhotra, Gelroth, Langemeier, and Rodgers (1995) evaluated the stability of β-carotene during baking and pre-baking processing steps for wheat-based products. The authors reported that baking caused significant reductions (74–85%) in the all-*trans* β-carotene isomer content. Similarly, Ranhotra et al. (1995) found that carotene losses were observed during baking, ranging from 4 to 15% for whole wheat bread products and from 18 to 23% for crackers. In another study, Leehardt et al. (2006) reported losses in total carotenoids of ~40% during bread baking. Highest losses occurred in the crust which experienced higher temperatures than the crumbs. The authors also reported that β-cryptoxanthin levels in bread crusts and water biscuits increased after baking whereas the levels of lutein, zeaxanthin, α- and β-carotene decreased. The same authors also found that the most significant decreases (66%) in total carotenoids content occur during kneading with a high correlation of these losses to the lipoxygenase activity of the wheat variety. Two separate studies reported decreases in the tocopherol and carotenoid content during the production of bread, water biscuits, and pasta from wheat flours (Hidalgo & Brandolini, 2010). Similarly, Lu, Fuerst et al. (2014) recently observed a decrease in the concentration of tocopherols and carotenoids during baking of two bread fractions (crumbs and upper crust). Recently, Kumar, Swathi, and Krishna (2014) investigated the effect of temperature on fat-soluble compounds (total tocopherols and tocotrienols, steryl ferulates, and carotenoids) in wheat bran and wheat germ. The authors found that the reduction of total tocopherols and tocotrienols, steryl ferulates, and carotenoids in wheat germ started at 130 °C.

Non-thermal processing, such as sprouting and kneading treatment, may also change the levels of phytochemicals in wheat samples. Alvarez-Jubete, Wijingaard, Arendt, and Gallagher (2010) reported that sprouting increased the total phenolic content in wheat from 53.1 to 110 mg GAE/100 g dwb. However, Leehardt et al. (2006) detected significant decreases (66%) in the total carotenoid content during kneading with a high correlation of these losses to the lipoxygenase activity of the wheat varieties. Furthermore, the same group reported a 10–12% loss in the tocopherol content during kneading (Leehardt et al., 2006). Changes in tocopherol and tocotrienol levels were also observed during dough making for bread, biscuit, and pasta (Hidalgo & Brandolini, 2010). The tocopherol content decreased by 21.4, 28.2, and 44.2% for bread, biscuit, and pasta, respectively.

5.3. *ARs during processing*

The processed grain products also vary in their ARs content. High temperature processing of grain based foods leads to denaturation or degradation of ARs (Weipert & Al Baya, 1977; Winata & Lorenz, 1997). Foods like white flour, corn, and rice-based grains, which do not contain whole wheat or wheat bran, showed low AR content (Holt, Moreau, DerMarderosian, McKeown, & Jacques, 2012). In a recent study by Gunenc et al. (2013), the authors evaluated the stability of alkylresorcinols in breads enriched in hard and soft wheat brans. The different formulas used in four bread trials were: A) 100% white flour (control bread), B) 30% wheat bran, C) 30% residue bran after AR extraction, and D) 30% wheat bran plus 2% crude AR extract. AR content in breads varied from 1.1 to 82.9 mg/100 g and was heat stable during baking. After 1 h cooling, all breads showed significantly different ($P < 0.05$) inner temperature, height and weight compared to the control bread. Furthermore, bread D showed a better height than breads B and C ($P < 0.05$). The effects of baking on total phenolic content (TPC) and antioxidant activity were also determined. A positive correlation was observed between oxygen radical absorbance capacity (ORAC) and TPC ($R^2 = 0.89$).

5.4. *Miscellaneous bioactive phytochemicals during processing*

In a study by Pedersen et al. (2011), ten benzoxazinoids were quantified in durum wheat before and after five-day hydrothermal processing (HTP) as well as in baked bread made with HTP and conventional flour. The authors reported significant concentration of benzoxazolinoids in HTP processed flour and no significant amount in conventional flour. However, the quantity of benzoxazolinoids in baked bread made from HTP flour was reduced by around five fold but increased marginally

in bread baked from conventional flour. Leaching of benzoxazinoids during soaking or boiling due to the enzymatic activity was reported by Tanwir et al. (2013). According to Kumar et al. (2014), processing reduces the levels of steryl ferulates which affect their antioxidant potentiality. In addition, Soupas, Huikko, Lampi, and Piironen (2007) suggested that the frying can affect the availability of phytosterols and reduces their levels due to oxidation. The lignans were not affected by moderate heat processing (100 °C), whereas high temperature processing (roasting at 250 °C) degraded the compounds. However, moderate heating enhancing the extractability of lignans was reported by Gerstenmeyer, Reimer, Berghofer, Schwartz, and Sontag (2013).

6. Bioactivity of wheat

There is an increased consumption in whole grain products because of their high functional property. Whole wheat grain products are enriched in bioactive phytochemicals such as phenolic acids, carotenoids, tocopherols, ARs, benzoxazinoids, phytosterols and lignans. Wheat phytochemicals are reported for their wide array of bioactivities namely, high free radical scavenging activity (Hosseinian & Mazza, 2009; Lu, Luthria et al., 2014; Lu, Lv et al., 2014; Lv et al., 2013), anticancer property (Liu, Winter, Stevenson, Morris, & Leach, 2012), reduction of cholesterol (Tiwari & Cummins, 2009), decreasing/controlling diabetes, etc. (Gil, Ortega, & Maldonado, 2011).

6.1. *Functional properties of phenolic compounds*

The wheat grains are rich in phenolic phytochemicals and are widely studied for their high antioxidant activity (Liyana-Pathirana & Shahidi, 2007; Okarter, Liu, Sorrells, & Liu, 2010). Phenolics like ferulic acid in wheat, behave as physical and chemical barriers to protect the kernel. This is achieved by combating destructive radicals and deters consumption by insects and animals because of its astringent taste. There is some evidence that these compounds promote resistance to various diseases. When compared to vegetables and fruits the free phenolic content of grains is less as most phenolic compounds exist in the bound form. Practically, the bran is not easy to digest by the human system and hence gastrointestinal esterase (from intestinal mucosa and microflora) facilitates the release of ferulic acid and di-ferulic acids from bran. The release of phenolic acids in the intestine reduces the risk of colon cancer in humans (Sang & Zhu, 2014). Nuclear factor kappa B (NF-kB) is a transcription factor regulating pro-inflammatory genes by controlling innate immunity processes, apoptosis, cell proliferation, and cell survival. Direct correlation between the increases in NF-kB activity was observed with several human cancers and in chronic inflammatory diseases (Hole, Grimmer, Jensen, & Sahlstrøm, 2012). The wheat phenolic acids (free and bound) were reported to modulate NF-kB activity.

6.2. *Functional properties of carotenoids and tocopherols*

Grains are one of the important sources of carotenoids and tocopherols. Nutritionally, carotenoids are beneficial to humans for lowering of LDL cholesterol levels by inhibiting cholesterol biosynthesis (Tiwari & Cummins, 2009). Carotenoids increase iron absorption in the human body by binding iron at the aromatic ring(s) and/or the conjugated double bond system (García-Casal, 2006). Tocopherols and tocotrienols are naturally occurring fat soluble components that protect biological membranes from oxidation and preserve immunological functions (Frank, Chin, Schrader, Eckert, & Rimbach, 2012). They play a potential role in reducing various degenerative diseases such as cardiovascular, cancer, inflammatory, neurological disorders, and cataracts.

6.3. *Bioactivity of ARs*

ARs can serve as *in vitro* antioxidant source due to their hydrogen donor and radical scavenging abilities (Gliwa, Gunenc, Ames, Willmore, & Hosseinian, 2011). Previous studies suggested that the membrane-located ARs are absorbed up to 80% by the human body (Kamal-Eldin, Pouru, Eliasson, & Aman, 2001). Liposomes are one of the effective drug carriers due to their ability to reduce drug toxicity and deliver the drugs to specific cells (Torchilin, 2005). The physicochemical behaviors of ARs and their variability in structures were reported for their suitability as effective drug delivery systems (Zant-Przeworska, Stasiuk, Gubernator, & Kozubek, 2010). The studies of Liu et al. (2012) suggested that the AR fractions such as 5-heptadecylresorcinol (IC_{50} = 22.5l g/mL), 5-(16-heneicosenyl) resorcinol (trans) (IC_{50} = 13.7l g/mL), 5-(14-nonadecenyl)resorcinol (trans) (IC_{50} = 42.2l g/mL), and 5-(2-oxotricosanyl) resorcinol (IC50 = 10.91 g/mL) showed high cytotoxicity against human prostate adenocarcinoma (PC3) cells at the IC_{50} concentration of 22.5, 13.7, 42.2 and 10.9 μg/mL (Landberg, Kamal-Eldin, Andersson, Vessby, & Åman, 2008). In a recent study, it was shown that the human plasma AR levels reach micro molar concentrations immediately after consuming whole grain wheat and rye product (Landberg et al., 2014). This indicates that the ARs were absorbed by the human body in a short time with a high efficiency. Landberg et al. (2014) revealed that the enzyme inhibition potential of ARs leads to the suppression of adipocyte lipolysis.

6.4. *Functional properties of miscellaneous bioactive phytochemicals*

In addition to above four classes of compounds, whole grains are known to possess other bioactive phytochemicals (benzoxazinoids, phytosterols, lignans, and etc.) in minor amounts. The benzoxazinoids can serve as biomarkers of whole grain as they are absorbed and metabolized by the body (Adhikari et al., 2013). Anti-inflammatory, anti-allergic, and anti-carcinogenic effects of individual benzoxazinoids were reported (Poupaert, Carato, & Colacillo, 2005). The main function of phytosterols is the regulation of membrane fluidity and permeability. In humans these compounds are known for their serum-cholesterol lowering effect (Kritchevskya & Chen, 2005). Lignans are claimed to have estrogen activity and hence served as phytoestrogens to humans (Aehle et al., 2011). A correlation between high intake of lignans and breast cancer was reported by Landete (2012). Lignans have also been reported to have anti-cancer activity against prostate cancer and

colorectal cancer (Landete, 2012). Higher levels of intake have been reported to reduce the risk of cardiovascular disease (Peterson et al., 2010).

7. Conclusions

Whole wheat grain and its derived food products provide a great source of bioactive phytochemicals that provide health benefits to humans. The bioactive phytochemicals are not uniformly distributed in grains; bran and germ provide higher concentrations of bioactive phytochemicals. A comparison of different technologies for extraction and analysis of bioactive phytochemicals from whole wheat is summarized in this review. It is clear that most phenolic acids are present in a bound form and improved yields are obtained after base hydrolysis using microwave, ultrasonication, and pressurized liquid extraction methods. Spectrophotometric determinations are used for quick total quantification; however, HPLC and GC methods coupled to mass spectrometers have been widely used for separation, identification, accurate quantification, and metabolic profiling. There has been increased interest in understanding how processing impacts the concentration of different bioactive phytochemicals present in whole wheat. NMR techniques have been used in a limited way for structural elucidation and profiling. There has been a large number of bioactivity reports on phytochemicals extracted from wheat. There is very limited research regarding interactions of different phytochemicals as they exit in wheat and wheat products. In addition, there is also limited research on increasing the bioavailability of bound phenolics.

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