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Colored grains: Chemistry, health benefits and processing

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Abstract
Grains are important part of our diet and among them colored grains are getting more research interest among scientist due to their enhanced nutritional, antioxidant and health promoting properties. According to different studies anthocyanins like cyanidin, malvidin, delphinidin, petunidin, peonidin and pelargonidin glycosides were identified and quantified in different colored grains like rice, maize, barley and wheat mainly by chromatographic and spectroscopic methods. Antioxidant activity of these pigmented grains cereals are measured using DPPH, FRAP, ORAC and ABTS assays and it was found to be related to the total anthocyanin content. Currently researches are being conducted on baked and extruded products from colored cereals like bread, biscuit, muffins, pasta, noodles etc. Application of colored grains are limited in to food colorants and in small scale as functional food products due to the thermal stability related issue of the bioactive compounds in colored grains and this area should be taken more in to consideration to stabilize these compounds and to introduce these pigmented grain based products to consumers in large scale after proper market analysis.

Keywords: Colored grains, anthocyanins, antioxidant, health benefit, food application

Introduction

Cereals are edible seeds of grass family, *Gramineae* which includes many crops like wheat, rice, maize, barley, oats, sorghum and millets etc. Among these cereals rice and wheat account for the 50% of world cereal production. According to FAO statistics (2021) [14] world cereal production and utilization is forecasted to be 2744.3 million tonnes and 2761.4 million tonnes respectively. Cereals are considered staple food both in developed and developing countries since they are considered as rich source of carbohydrate, protein and dietary fiber and micronutrients such as vitamin E, some of B group vitamins, magnesium and zinc. The wide acceptability of cereal is due to the fact that they are inexpensive to produce, easy to store, easy to transport, and they do not deteriorate readily if kept in dry conditions (McKevith, 2004) [42].

Nature contain more than 5000 flavonoids, among them sap- soluble flavonoids are responsible for imparting color to the plants. Anthocyanins are an important class of flavonoids that are water-soluble glycosides of polyhydroxy and polymethoxy derivates of 2-phenylbenzopyrylium or flavylium salts that provide red, purple, blue and black color to many plant parts (Zilic *et al.*, 2012) [80]. Anthocyanins occur in nature as glycosides where the anthocyanidin compound is not coupled with sugar molecule. Anthocyanins are classified based on the modifications like substituent groups on the B ring, type and number conjugated sugar and also based on the presence or absence of an acyl group. Pelargonidins, cyanidins, delphinidins, peonidins, petunidins, and malvidins are the six principle anthocyanins in nature. The abundance of anthocyanins varies according to plant species and the time of harvest and these bioactive compounds are found in many plant foods like cereals, vegetables, fruits, pulses, tubers and roots (Tsuda, 2012) [70]. In colored grains like rice, maize, barley and wheat anthocyanin is responsible for pigmentation that result in black, blue, purple, red and combination of these colors in them (Garg *et al.*, 2016; Žilić *et al.*, 2012; Kim *et al.*, 2008; Kim *et al.*, 2007) [19, 80, 31, 30].

Cereals are rich source of carbohydrates, proteins, B group vitamins and minerals and also contain phytochemicals or plant bioactive compounds responsible for providing health benefits. Epidemiological studies on whole grains indicate that regular consumption of whole grains and wholegrain products helps to reduced risks of chronic diseases such as cardiovascular diseases (Okarter *et al*., 2010) [47] type 2 diabetes (Tapola *et al*., 2005) [68] and some cancers (Haas *et al*., 2009) [23]. Bioactive compounds in pigmented cereals possess high antioxidant and antiradical activities, antimutagenesis, anticarcinogenesis, estrogenic activities, inhibition of enzymes and induction of detoxification enzymes (Adom *et al*., 2002) [7]. Anthocyanin rich foods and the pigment itself is helpful to reduce the risk of colon cancer by inhibiting proliferation of human colon cancer cells *in vitro* (Jing *et al*., 2008) [29] and according to Tsuda *et al*., 2003 [72] anthocyanin is helpful in preventing diabetes and obesity.

This review paper contains detailed information about pigmented rice, wheat, maize and barley. Here the discussion is about the bioactive compounds, antioxidant potential, health benefits, application in food industry and thermal stability of the bioactive compounds in colored grains.

Types of colored grains

This review paper contains information about four types of colored cereals that are rice, maize, barley and wheat and the pigmentation is mainly due to the anthocyanin produced by flavonoid biosynthetic pathway that can be better understood by Fig 1.

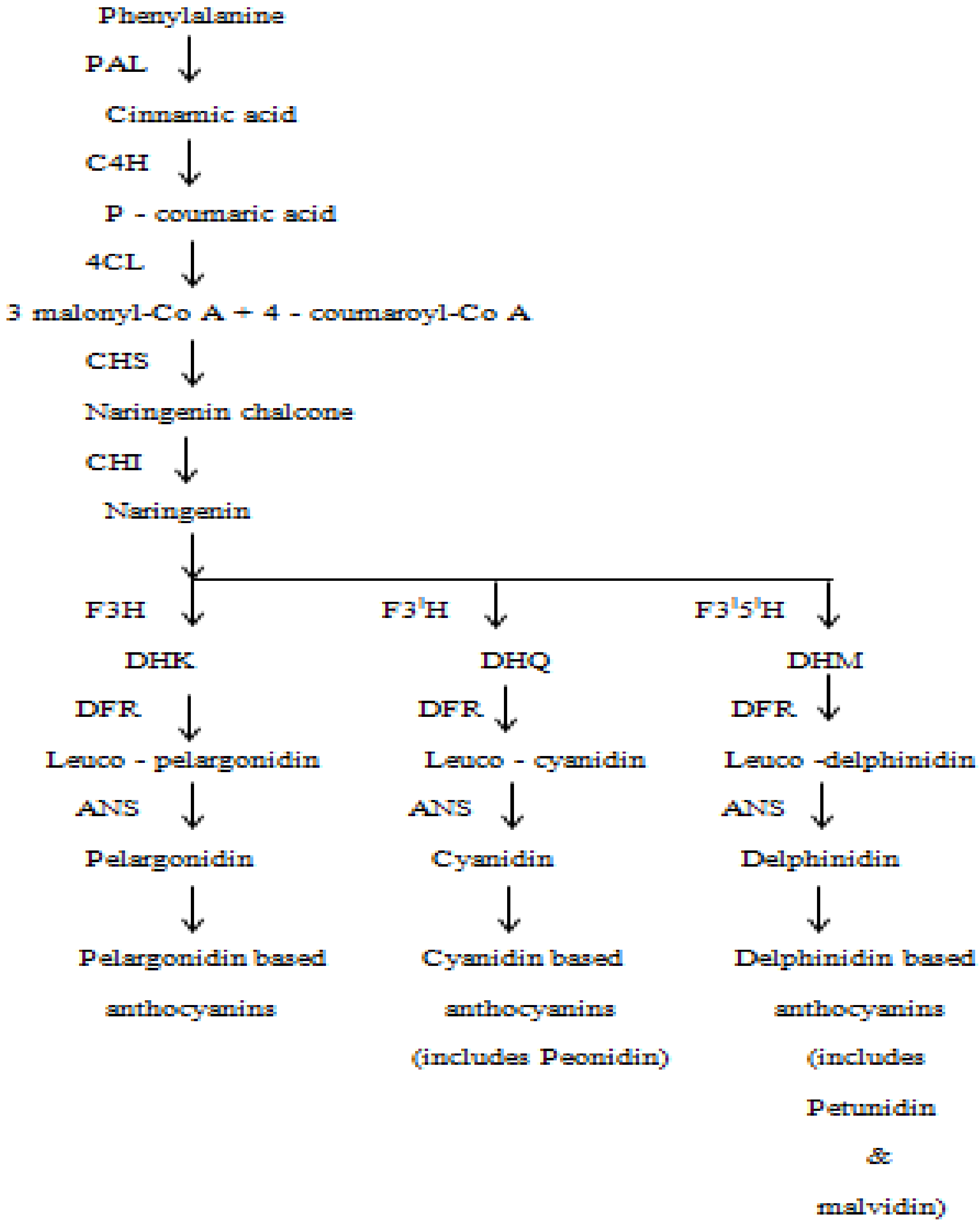

Fig 1: Flavonoid biosynthesis pathway (Ng and Smith, 2016; Ravaglia *et al*., 2013) [46, 52].

PAL: phenylalanine ammonia-lyase; C4H: cinnamate 4-hydroxylase; 4CL: 4-coumarate-CoA ligase; CHS: Chalcone Synthase; CHI: Chalcone Isomerase; F3H: Flavanone 3-hydroxylase; F3′ H: flavonoid 3′ -hydroxylase; F3′ 5′ H: flavonoid 3′, 5′ -hydroxylase; DHK, dihydrokaempferol; DHM: dihydromyricetin; DHQ: dihydroquercetin; DFR: Dihydro Flavonol-4-Reductase; ANS: Anthocyanidin Synthase

As per the different research works conducted on colored grains black, blue, purple and red color of the cereal is proportional to the concentration of anthocyanin present in them.

Rice

Rice is the principle cereal for most of the people in Asia and also considered as a staple food for half of world population. Rice is available in white, brown and colored forms and the consumption of colored varieties is becoming popular because of their proved health benefits. Colored rice varieties are rich in polyphenols that possess health promoting properties by reducing the concentration of reactive cell-damaging free radicals. Anthocyanin is the flavonoid responsible for red, purple, blue and black color in rice varieties. These compounds are found to have health promoting properties including antioxidant, anti-cancerous, anti-inflammatory properties and hypoglycemic properties. The consumption of colored rice is becoming popular worldwide where the black rice is often mixed with non-colored rice before cooking to improve the flavor characteristics (Yoshida *et al*., 2010) [75] and red colored rice is used as a food colorant in bread, ice-cream and liquors (Abdel-Aal *et al*., 2006) [6].

Maize

Maize is a widely cultivated cereal in the world that is considered as an integral part of diet for all socioeconomic class around the world. The pigmented maize kernels are only produce in small quantity around the world to be used in ornamentation and to make specialty food due to the appearance. Colored maize contains many secondary metabolites like phenolic compounds and carotenoids. Among the phenolic compounds, Phenolic acids and flavonoids are the most common form found in whole maize kernel in soluble free or insoluble bound form. Flavonoid compounds are responsible for most orange, scarlet, crimson, mauve, violet, and blue colors, as well as contributing yellow, ivory, and cream colors to the maize varieties. Among them anthocyanins in Simple or acylated form mainly located in the aleurone layer or pericarp of the maize endosperm greatly affecting the color of the kernel that could be red, purple, blue or black (Zilic *et al*., 2012) [80].

Barely

Barley is a crop used for human consumption, animal feed and industrial applications that can be cultivated in a wide range of climates around the world. Barley is considered as a source of carbohydrate but other than that it also contains protein, fat, crude fiber, calcium and phosphorus (Baik *et al*., 2008) [8]. Colored barley kernel contains anthocyanin, carotenoids, phenolic acids and other phytochemicals produced by secondary metabolism. Pigmentation of barley such as black, blue, red, and purple varieties are due to the presence of both melanins and anthocyanins. According to different studies these compounds are found to be potent antioxidants, free radical scavengers, and metal chelators, and help to inhibit lipid peroxidation (Shen *et al*., 2018; Nascimento *et al*., 2018; Rocchetti *et al*., 2017; Shen *et al*., 2016; Fattore *et al*., 2016) [60, 45, 53, 61, 15].

Wheat

Wheat is the highest cultivated and consumed grain in the world. Anthocyanin is the reason for black, blue and purple color of wheat kernels depending on their concentration while the red color is due to the presence of compound called phlobaphene and yellow color is due to the presence of carotenoids. The concentration of anthocyanin is highest in black colored wheat grain and the concentration gradually reduces in the order of blue-purple-red-amber colored wheat, respectively (Sharma *et al*., 2018) [58]. Health benefits of colored wheat grains like high antioxidant activity, neuroprotection, inhibition of cholesterol absorption, anticancer action, retinal protection, anti-aging, immune boosting and anti- hypertension effects are due to the presence of these bioactive compounds in them. Colored wheat varieties possess high nutritional content and antioxidant activity considered to non-pigmented wheat varieties hence can be used for the production of products like bread, biscuit, pasta, noodles and crackers to substitute normal wheat (Saini *et al*., 2020) [54].

Bioactive compounds in colored grains

In order to identify and quantify the amount of anthocyanins in colored grains chromatographic and spectroscopic methods are preferred usually. Among these anthocyanins cyanidin, delphinidin, petunidin, peonidin, malvidin and pelargonidin related glucosides are identified and quantified in colored grains.

Abdel-aal *et al*., 2006 [6] identified and studied purity of anthocyanins in colored grains using liquid chromatography-ultraviolet-visible spectroscopy and liquid chromatography-mass spectrometry. In case of black and red rice Cyanidin 3-glucoside was the most abundant anthocyanin, while Peonidin 3-glucoside was second abundant and cyanidin diglucoside was the third major anthocyanin in both rice grains. In case of blue wheat grain delphinidin 3-glucoside was the first abundant anthocyanin followed by delphinidin3-rutinoside. In purple wheat 10 anthocyanins were identified in small concentration and among them cyanidin 3-glucoside and peonidin malonylglucoside were the main ones. Only two anthocyanins were detected in blue barley and these are cyanidin-3-glucoside and petunidin-3-glucoside. Blue, pink, purple, red and multi colored corn exhibited complex anthocyanin composition starting from 18 to 27 compounds. Cyanidin 3-glucoside was the most common anthocyanin in colored corn, except for pink corn where the most abundant anthocyanin was pelargonidin 3-glucoside. In case of corn second most abundant anthocyanin was different in corn, being cyanidin 3-succinylglucoside in blue, purple, and multicolored corn, peonidin 3-glucoside in red corn and cyanidin 3-glucoside in pink corn.

In the research work conducted by Abdel-aal *et al*., 2014 [3] on blue wheat, purple corn and black rice it was found that the anthocyanin extracted in total and their composition were affected differently by the method of extraction and type of solvent because the anthocyanin profile of the grains are different. Different methods for extraction of anthocyanins from colored grains are mentioned in Fig 2. In this study Changes to the blue wheat anthocyanins were less compared to purple corn and black rice due to the absence of acylated anthocyanins in purple corn and black rice. Changes in the amount of anthocyanins and their composition of the final extract will affect their antioxidant properties, use as a food colorant and eventually affect their health promoting properties due to the impact of structure on intensity of color, antioxidant activity. For commonly used solvent extraction (CSE) acidified methanol with HCl, acidified methanol with phosphoric acid, acidified methanol with trifluoro acetic acid, acidified methanol with glacial acetic acid, and acetone/water were used for extraction purpose. In case of accelerated solvent extraction (ASE) and microwave assisted extraction (MAE) method organic or phosphoric acid was used to replace HCl due its degradation effect on acylated anthocyanins particularly at elevated temperature and/or pressure and this can also be helpful to avoid the corrosive effect of HCl in ASE or MAE extractors. According to this research accelerated solvent extraction (ASE) method found to be more appropriate for the extraction of anthocyanins from colored grains on comparison with commonly used solvent extraction (CSE) method and ASE method also showed lower changes in the anthocyanin content and composition on comparison with microwave assisted extraction (MAE) method.

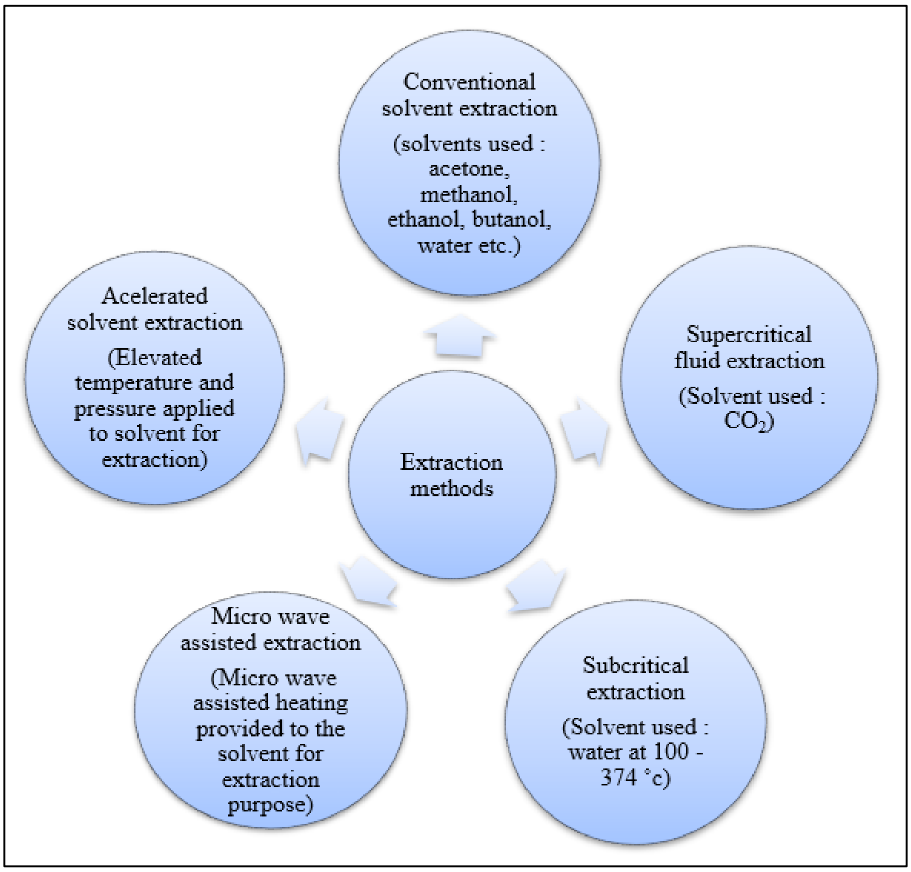

Fig 2: Different methods for extraction of anthocyanins from colored grains (Abdel- aal *et al*., 2014; Limtrakul *et al*., 2019) [3, 36].

Rice

Seo *et al*., 2011 [57] conducted a study on the 12 Korean colored rice varieties Heugnam, Sintoheugmi, Hongjinju, Jeogjinju, Heugjinju, Boseogheugchal, Sinnongheugchal, Sinmyungheugchal, Josaengheugchal, Heuggwang, Heughyang and Heugseol. In this study total phenolics, protein, lipid and total anthocyanin content of these rice varieties were evaluated in order to understand their antioxidant potential in a better way. Three anthocyanins, cyanidin-3-O-glucoside (Cy-3-G), petunidin-3-O-glucoside (Pt-3-G) and peonidin-3-O-glucoside (Pn-3-G) were also characterized by ultra-performance liquid chromatography. Among the rice varieties, Heugjinju (1338.58 mg CGA/100g), Heugseol (1173.27 mg CGA/100g), and Sintoheugmi (1119.43 mg CGA/100g) contained high total phenolic content and the lowest phenolic content was detected in Jeogjinju (221.00 mg CGA/100g). Total anthocyanin content was highest in Heugjinju (122.65 mg/100g) and no anthocyanins were detected in Hongjinju and Jeogjinju. To determine the relation between total polyphenolic and anthocyanin contents to antioxidant activity the study focused on linear regression analysis. According to the results total polyphenolic content was strongly correlated with antioxidant activity, thus it can be considered as key factor in antioxidant activity of colored rice. Among the colored rice varieties Heugjinju, Heugseol, and Sintoheugmi varieties also possess important nutritional value considered to other colored varieties.

Kim *et al*., 2008 [31] studied about the anthocyanins from black, red and wild varieties of rice. Extraction of anthocyanin was conducted using acidified methanol with 0.1M HCl as the solvent, separation and identification of the anthocyanins were done using reverse phase HPLC followed by detection using ultraviolet-visible absorption spectrophotometer and further identification using LC- MS. Black and wild rice varieties had three different types of pigments detected by HPLC and among them one was characterized as cyanidin-3-glucoside (C3G) on comparison of spectroscopic and chromatographic properties with standard, and another one among the three was tentatively identified as cyanidin-fructoside and the one last pigment was not identified. As per the study most abundant anthocyanin in black (2.1 mg/g) and wild rice (3.3 mg/g) was cyanidin-3-glucoside. No anthocyanin was detected in red variety using HPLC. After the confirmation of the anthocyanin in the unknown peak, in order to identify the sugar moiety present in the colored rice varieties HPLC chromatography was performed after acid hydrolysis. The result indicates that the sugar moieties in black rice are fructose (3.5µg/g) and glucose (5.5µg/g). Different types of anthocyanins and their quantities in colored rice grains are mentioned in Table 1.

Table 1: Different types of anthocyanins in colored rice grains

Colored grain	Type of anthocyanin	Quantity (mg/kg)	References
Black rice	Cyanidin-3-glucoside	88.6- 2013	Seo *et al*., 2011 [57] Sompong *et al*., 2010 [63] Abdel – aal *et al*., 2006 [6]
	Petunidin-3-glucoside	4 – 10	Seo *et al*., 2011 [57]
	Peonidin-3-glucoside	135 – 1275	Seo *et al*., 2011 [57] Sompong *et al*., 2010 [63]
	Cyanidin-3-rutinoside	19.90	Abdel – aal *et al*., 2006 [6] Huang and Lai, 2016 [27]
Purple rice	Cyanidin-3-glucoside	10 – 1170	Chen *et al*., 2017 [11]
Red rice	Cyanidin-3-glucoside	0.245 – 14	Samyor *et al*., 2017 [56] Pereria-Caro *et al*., 2013 [50] Abdel – aal *et al*., 2006 [6] Frank *et al*., 2012 [17]
	Cyanidin-3-rutinoside	1.30	Abdel – aal *et al*., 2006 [6] Huang and Lai, 2016 [27]
	Peonidin-3-glucoside	0.082 - 2.5	Abdel – aal *et al*., 2006 [6] Frank *et al*., 2012 [17]

As per the research conducted by Sompong *et al*., 2010 [63] on black, red and wild rice varieties, the major phenolic acids in the free form in red rice varieties were ferulic, protocatechuic and vanillic acid and in case of black rice varieties protocatechuic acid was dominant followed by the vanillic and ferulic acid. For the bound form of phenolic acid ferulic acid was dominant in both colors but the contents differed significantly followed by p-coumaric and vanillic acid.

Maize

Zilic *et al*., 2012 [80] studied about the total flavonoids, phenolics, anthocyanin, free, conjugated, and insoluble bound phenolic acids, β-carotene and lutein in 10 different colored maize genotypes such as white, yellow, orange, red- yellow, red, blue and multicolored etc. Total flavonoid content of the colored maize varieties varied from 248.6 mg CE/ kg dry matter to 337.51 mg CE/ kg dry matter. The β-carotene and lutein content of the varieties ranged from 0 to 2.42 mg/kg by dry matter basis and from 0 to 13.89 mg/kg dry matter basis respectively. Total anthocyanin contents of colored maize genotypes ranged from 2.50 to 696.07 mg CGE/kg dry matter (cyanidin 3-glucoside equivalent), were cyanidin-3- glucoside (0.07 mg/ kg dry matter to 547.49 mg/kg dry matter) was the most dominant form. The light blue maize genotype had higher content of total phenolics, flavonoids, and ferulic acid as compared to other tested colored maize varieties. It can be concluded that the orange colored genotype has a superior potential to be used as a source of pro-vitamin A. According to the results of total anthocyanin contents colored maize kernels such as dark red, dark blue, and light blue maize can be used for the development of functional foods and/or natural colorants due to the high anthocyanin content.

Salinas Moreno *et al*., 2005 [55] characterized the pigments present in Arrocillo, Cónico, Peruano, and Purepecha varieties of maize to understand their potential use as natural dyes. These four varieties have intense purplish-red kernels and pigment is present in both pericarp and aleurone layer. Determination of total anthocyanin content was done by conventional spectrophotometric method and the characterization of the anthocyanins was done by high-performance liquid chromatography. According to the results, maize samples contained anthocyanin in both the aleurone and pericarp layer. Total anthocyanin content ranged from 54 mg/100 g to 115 mg/100 g in the degermed kernel. In case of the pericarp portion of the kernel total anthocyanin ranged from 504 mg/100g to 1473 mg/100g. In the endosperm portion of the kernel anthocyanin content was between 4.2 mg/100g to 26.8 mg/100g. Anthocyanin profiles are almost the same among the four maize samples and small differences was observed only in the relative percentage of each anthocyanin. The identified anthocyanins are cyanidin-3-glucoside, pelargonidin- 3-glucoside, peonidin-3-glucoside, cyanidin-3-(6” malnoylglucoside) and cyanidin-3-(3”,6” dimalonylglucoside). The type of anthocyanins and their quantities in colored maize are given in Table 2.

Table 2: Different types of anthocyanins in colored maize

Colored grain	Type of anthocyanin	Quantity (mg/kg)	References
Purplish black maize	Cyanidin-3-glucoside	380.35	Harakotr *et al*., 2014 [24]
	Pelargonidin- 3- glucoside	98.79	Harakotr *et al*., 2014 [24]
	Peonidin – 3- glucoside	133.05	Harakotr *et al*., 2014 [24]
Blue maize	Cyanidin-3-glucoside	308.24 – 312.43	Zilic *et al*., 2012 [80] Abdel-aal *et al*., 2006 [6]
	Pelargonidin- 3- glucoside	1.34 – 12.1	Zilic *et al*., 2012 [80] Abdel-aal *et al*., 2006 [6]
	Cyanidin - 3,5 – di glucoside	1.36 – 1.65	Zilic *et al*., 2012 [80]
Purple maize	Cyanidin-3-glucoside	70 – 961	Zhang *et al*., 2019 [76]

			Abdel-aal *et al*., 2006 [6]
	Pelargonidin- 3- glucoside	20 – 3390	Zhang *et al*., 2019 [76] Abdel-aal *et al*., 2006 [6]
	Peonidin – 3- glucoside	60 – 2050	Zhang *et al*., 2019 [76] Abdel-aal *et al*., 2006 [6]
Red maize	cyanidin-3-O-glucoside	284.5 – 7000	Mazewski *et al*., 2017 [41] Abdel-aal *et al*., 2006 [6]
	Pelargonidin -3-O-glucoside	27.7	Abdel-aal *et al*., 2006 [6]
	Peonidin- 3- o- glucoside	58.7 – 2700	Mazewski *et al*., 2017 [41] Abdel-aal *et al*., 2006 [6]
Pink maize	Cyanidin-3-glucoside	3.44 – 7.8	Harakotr *et al*., 2014 [24] Abdel-aal *et al*., 2006 [6]
	Pelargonidin- 3- glucoside	7.81- 39.8	Harakotr *et al*., 2014 [24] Abdel-aal *et al*., 2006 [6]
	Peonidin – 3- glucoside	0.68 – 0.7	Harakotr *et al*., 2014 [24] Abdel-aal *et al*., 2006 [6]

Barley

Ge *et al*., 2021 [20] evaluated the phenolic content and antioxidants activities of white, yellow, black and blue naked barley. According to the study 156 phenolic substances were identified like monophenol, phenolic acids, flavonoids and other polyphenols. From the study it was clear that the phenolic content depends on the color of the naked barley and thus total flavonoid and phenolic acid was highest in black barley. As per the research 73, 76, 86 and 89 phenolic compounds were detected in the white, yellow, blue and black naked barley samples, respectively. Among these phenolic compounds 28 were found in all the colored naked barley and that includes 2-hydroxyadenosine, 4-hydroxybenzoic acid, astragalin, calceorioside B, luteolin-7-O-β-D-glucoside, luteoloside etc. The compounds such as butylparaben, curcumin and isorhamnetin were identified only in white naked barley. In case of black naked barley 20 compounds were unique to them and that includes 4-hydroxycoumarin, 7-hydroxycoumarin, chlorogenic acid, daidzin, dihydroquercetin, eriodictyol, farrerol, icaritin, neochlorogenic acid, and scutellarin etc. For yellow barley 15 phenolic compounds were exclusive for them and that includes 6-methylcoumarin, baicalein, carnosic acid, daphnetin, esculetin, isoliquiritigenin, liquiritigenin, pinocembrin, resveratrol and salidroside etc. For blue barley 18 compounds unique to them and that includes (+)-protosappanin B, astilbin, carvacrol, corylifol A, dihydromyricetin, glycitin, naringin and schaftoside etc.

Suriano *et al*., 2018 [64] conducted a study on 20 genotypes of colored barley including 16 F8 recombinant inbred lines (RILs) and their four parental lines i.e. 2005 FG (blue and naked seeds), K4-31 (black and hulled), L94 (black and naked) and Priora (white and naked).Among the inbred lines the cross of parental lines K4–31 × Priora × 2005FG were called B group that included 1956, 1960, 1966, 1981, 1997, 2000, 2002 and the cross of Priora × K4–31 × 2005FG were called C group that included 3001, 3002, 3003, 3004, 3005 3006, 3007, 3008, 3009. The objective of the study was to find out the free, soluble conjugated and insoluble bound phenolic acids and some of the main antioxidant phytochemicals such as total polyphenols, proanthocyanidins, and carotenoids in the colored barley varieties. It was observed that large variations were seen in the contents of phytochemicals and β-glucans across these colored barley genotypes. According to the results highest protein (14.4%) and β-glucan (4.6%) contents were in the blue parental naked genotype 2005 FG. Total polyphenols and proanthocyanidins were highest in the inbred lines 3009 (2917 µg GAE/g) and 1997 (1630 µg CA/g) respectively. Highest carotenoid content was observed in the parent line K4–31 i.e. 4.60 µg /g. After the HPLC analysis for phenolic acids in the colored barley varieties nine phenolic acids were defined in the barley and they are gallic, 3, 4-hydroxybenzoic, chlorogenic, caffeic, syringic, p-coumaric, ferulic, cinnamic and salycilic acids. The highest and lowest phenolic acid contents were observed in the inbred lines 3009 (513.5 µg /g) and 3004 (211.2 µg/g) respectively. Ferulic acid was the main conjugated phenolic acid detected. Salicylic and gallic acids were the highest frephenolic acids found and no p-coumaric and cinnamic acids detected in free form during the study. In case of insoluble bound phenolic acids it represented 88.3% of the total phenolic acids and ferulic acid was the most abundant bound phenolic compound found in the colored barley varieties. Different type of anthocyanins in colored barley grains such as cyanidin, peonidin, petunidin, malvidin, delphinidin etc. and their quantities are also mentioned in Table 3 for better understanding.

Table 3: Different types of anthocyanins in colored barley grains

Colored grain	Type of anthocyanin	Quantity (mg/kg)	References
Black barley	Delphinidin-3-glucoside	22.3 – 1000	Kim *et al*., 2007 [30] Suriano *et al*., 2019 [65]
	Cyanidin-3-glucoside	8.6 – 21.2	Kim *et al*., 2007 [30]
	Pelargonidin- 3- glucoside	12.1 – 20.3	Kim *et al*., 2007 [30]
	Peonidin – 3- glucoside	3.3 – 5.6	Kim *et al*., 2007 [30]
	Malvidin-3-glucoside	0.6 – 3920	Kim *et al*., 2007 [30] Suriano *et al*., 2019 [65]
Blue barley	Cyanidin-3-glucoside	1.2 – 290	Abdel-aal *et al*., 2006 [6] Suriano *et al*., 2019 [65]
	Petunidn- 3- glucoside	2.9	Abdel-aal *et al*., 2006 [6]
	Malvidin-3-glucoside	22830	Suriano *et al*., 2019 [65]
	Delphinidin-3-glucoside	3700	Suriano *et al*., 2019 [65]

	Peonidin – 3- glucoside	420	Suriano *et al*., 2019 [65]
	Delphinidin-3-rutinoside	470	Suriano *et al*., 2019 [65]
Purple barley	Delphinidin-3-glucoside	22.5- 93	Kim *et al*., 2007 [30] Bellido and Beta, 2009 [10]
	Cyanidin-3-glucoside	99 – 239.3	Kim *et al*., 2007 [30] Bellido and Beta, 2009 [10]
	Pelargonidin- 3- glucoside	42.2	Kim *et al*., 2007 [30]
	Peonidin-3- glucoside	32.7	Kim *et al*., 2007 [30]
	Malvidin-3-glucoside	13.7	Kim *et al*., 2007 [30]
	Petunidn- 3- glucoside	37	Kim *et al*., 2007 [30] Bellido and Beta, 2009 [10]

Wheat

Review by Lachman *et al*., 2017 [34] suggests that cereal grains consist of many phytochemicals and among them some can significantly influence the grain color. Anthocyanins are pigments mainly found in the aleurone or pericarp layer of the cereals and provide black, blue, purple or combination of these colors. Flavonoids such as C-glycosides of flavones, flavonols, flavanonols, proanthocyanidins and reddish-colored phlobaphenes are mainly present in the outer layer of grains while carotenoids that are responsible for yellow grain are in the endosperm. The difference between blue aleurone and purple pericarp is the presence of delphinidin-3-glucoside, delphinidin-3-rutinoside and malvidin-3-glucoside in blue aleurone wheat. In blue wheat cv. Purendo four main anthocyanins delphinidin-3-glucoside (45%), cyanidin-3-glucoside (28%), delphinidin-3-rutinoside (22%), and cyanidin-3-rutinoside (2%) were identified (Abdel-Aal *et al*., 2008) [2]. In case of purple wheat grain abundant anthocyanin is cyanidin-3-glucoside (Abdel-Aal and Hucl, 2003) [1] followed by cyanidin-3-galactoside and malvidin-3-glucoside (Hosseinian *et al*., 2008) [26]. Red wheat varieties does not contain anthocyanin pigments based on different studies conducted, but a small amounts of anthocyanin glycosides was reported according to the work by Abdel-Aal *et al*., 2006 [6]. In case of red durum wheat cyanidin-3-glucoside was the major component (4.02 mg/kg) present, followed by peonidin-3-galactoside (0.33 mg/kg) and malvidin-3-glucoside (0.22 mg/kg) (Ficco *et al*., 2014) [16].

Sytar *et al*., 2018 [67] conducted a study on blue, purple and yellow colored wheat grains and their sprouts to identify the bioactive phytochemicals and their antioxidant properties. Presence of total phenolics, flavonoids, anthocyanins and anthocyanidins (pelargonidin, peonidin, cyanidin, delphinidin) content was measured spectrophotometrically and using high performance thin layer chromatography (HPTLC). Types of anthocyanins in colored wheat grains along with their quantity are given in Table 4. According to the results a

Table 4: Different types of anthocyanins in colored wheat grains

Colored grain	Type of anthocyanin	Quantity (mg/kg)	References
Black wheat	cyanindin-3-o-glucoside chloride	20.50	Sharma *et al*., 2020 [58]
	Delphinidin-3-o-glucoside chloride	25.64	Sharma *et al*., 2020 [58]
	Petunidin-3-o-glucoside chloride	2.29	Sharma *et al*., 2020 [58]
	Peonidin-3-o-glucoside chloride	1.40	Sharma *et al*., 2020 [58]
	Malvidin-3-o-glucoside chloride	2.18	Sharma *et al*., 2020 [58]
	Pelargonidin 3-o-glucoside chloride	2.13	Sharma *et al*., 2020 [58]
Blue wheat	Cyanidin-3-glucoside	1.17 - 28.14	Gamel *et al*., 2020 [18] Zilic *et al*., 2019 [79] Garg *et al*., 2016 [19] Bartl *et al*., 2015 [9] Ficco *et al*., 2014 [16] Knievel *et al*., 2009 [32] Abdel-Aal *et al*., 2006 [6] Abdel-Aal and Hucl, 2003 [1]
	Cyanidin-3-rutinoside	7.12 - 16.8	Ficco *et al*., 2014 [16] Knievel *et al*., 2009 [32] Abdel-Aal *et al*., 2006 [6]
	Delphinidin-3-glucoside	0.52 - 56.5	Garg *et al*., 2016 [19] Bartl *et al*., 2015 [9] Ficco *et al*., 2014 [16] Knievel *et al*., 2009 [32] Abdel-Aal *et al*., 2006 [6]
	Delphinidin-3-rutinoside	31.77 - 49.6	Abdel-Aal *et al*., 2018 [5] Garg *et al*., 2016 [19] Bartl *et al*., 2015 [9] Ficco *et al*., 2014 [16] Knievel *et al*., 2009 [32] Abdel-Aal *et al*., 2006 [6]
	Malvidin-3-glucoside	10.26 - 13.8	Garg *et al*., 2016 [19] Ficco *et al*., 2014 [16] Hosseinian *et al*., 2008 [26] Abdel-Aal and Hucl, 2003 [1]
	Malvidin-3-rutinoside	2	Abdel-Aal *et al*., 2018 [5] Garg *et al*., 2016 [19]

			Bartl *et al*., 2015 [9] Abdel-Aal *et al*., 2006 [6]
	Peonidin-3-arabinoside	0.95 - 3.49	Ficco *et al*., 2014 [16] Hosseinian *et al*., 2008 [26]
	Peonidin-3-glucoside	0.76 - 0.99	Ficco *et al*., 2014 [16]
	Peonidin-3-galactoside	1.94	Ficco *et al*., 2014 [16] Abdel-aal and Hucl, 2003 [1]
	Peonidin-3-rutinoside	0.40 - 1.2	Giordano *et al*., 2017 [21] Garg *et al*., 2016 [19] Bartl *et al*., 2015 [9] Abdel-Aal *et al*., 2006 [6]
	Petunidin-3-glucoside	2.2	Garg *et al*., 2016 [19] Abdel- Aal *et al*., 2006 [6] Abdel-Aal and Hucl, 2003 [1]
	Petunidin-3-rutinoside	4.5	Abdel-Aal *et al*., 2006 [6]
Purple wheat	Cyanidin-3-arabinoside	23.2 - 25.1	Hosseinian *et al*., 2008 [26]
	Cyanidin-3-galactoside	0.98 - 72.0	Hosseinian *et al*., 2008 [26] Abdel-Aal and Hucl, 2003 [1]
	Cyanidin-3-glucoside	0.63 - 116.2	Gamel *et al*., 2020 [18] Zilic *et al*., 2019 [79] Garg *et al*., 2016 [19] Bartl *et al*., 2015 [9] Ficco *et al*., 2014 [16] Knievel *et al*., 2009 [32] Abdel-Aal *et al*., 2006 [6] Abdel-Aal and Hucl, 2003 [1]
	Cyanidin di-glucoside	1.7	Abdel-Aal *et al*., 2018 [5] Ficco *et al*., 2014 [16]
	Cyanidin-3-rutinoside	3.2 - 85.1	Ficco *et al*., 2014 [16] Knievel *et al*., 2009 [32] Abdel-Aal, *et al*., 2006 [6]
	Cyanidin chloride	5.2 - 6.8	Bartl *et al*., 2015 [9] Hosseinian *et al*., 2008 [26]
	Cyanidin-3-(6’-malonylglucoside)	1.2 - 31.08	Zilic *et al*., 2019 [79] Garg *et al*., 2016 [19] Abdel-Aal *et al*., 2006 [6]
	Cyanidin-3-(3’,6’-dimalonylglucoside	3.58 - 19.07	Zilic *et al*., 2019 [79] Garg *et al*., 2016 [19]
	Cyanidin-3-succinyl-glucoside	0.6 - 1.2	Garg *et al*., 2016 [19] Knievel *et al*., 2009 [32] Abdel-Aal *et al*., 2006 [6] Abdel-Aal and Hucl, 2003 [1]
	Delphinidin-3-arabinoside	15.1 - 16.7	Hosseinian *et al*., 2008 [26]
	Delphinidin-3-galactoside	38.3–36.4	Hosseinian *et al*., 2008 [26]
	Delphinidin-3-rutinoside	3.7	Abdel-Aal *et al*., 2018 [5] Garg *et al*., 2016 [19] Bartl *et al*., 2015 [9] Ficco *et al*., 2014 [16] Knievel *et al*., 2009 [32] Abdel-Aal *et al*., 2006 [6]
	Delphinidin-3-(6’-malonylglucoside)	3.5	Abdel-Aal *et al*., 2018 [5] Garg *et al*., 2016 [19]
	Malvidin-3-glucoside	0.17 - 60.3	Garg *et al*., 2016 [19] Ficco *et al*., 2014 [16] Hosseinian *et al*., 2008 [26] Abdel-Aal and Hucl, 2003 [1]
	Malvidin-3-rutinoside	2.6	Abdel-Aal *et al*., 2018 [5] Garg *et al*., 2016 [19] Bartl *et al*., 2015 [9] Abdel-Aal *et al*., 2006 [6]
	Pelargonidin-3-arabinoside	9.3 - 11.3	Lachman *et al*., 2017 [34] Hosseinian *et al*., 2008 [26]
	Pelargonidin-3-galactoside	22.1 - 26.1	Hosseinian *et al*., 2008 [26]
	Pelargonidin-3-glucoside	2.185 - 29.3	Zilic *et al*., 2019 [79] Garg *et al*., 2016 [19] Bartl *et al*., 2015 [9] Hosseinian *et al*., 2008 [26]
	Pelargonidin-3-(6’-malonylglucoside)	1.2	Zilic *et al*., 2019 [79] Abdel- Aal *et al*., 2018 [5] Garg *et al*., 2016 [19]
	Peonidin-3-arabinoside	28.4 - 29.3	Ficco *et al*., 2014 [16]

			Hosseinian *et al*., 2008 [26]
	Peonidin-3-glucoside	2.7 - 17.5	Gamel *et al*., 2020 [18] Garg *et al*., 2016 [19] Bartl *et al*., 2015 [9] Ficco *et al*., 2014 [16] Hosseinian *et al*., 2008 [26] Abdel-Aal *et al*., 2006 [6] Abdel-Aal and Hucl, 2003 [1]
	Peonidin-3-galactoside	0.06 - 3.11	Ficco *et al*., 2014 [16] Abdel- Aal and Hucl, 2003 [1]
	Peonidin-3-rutinoside	9.36	Giordano *et al*., 2017 [21] Garg *et al*., 2016 [19] Bartl *et al*., 2015 [9] Abdel-Aal *et al*., 2006 [6]
	Peonidin malonyl-glucoside	0.6	Abdel-Aal *et al*., 2006 [6]
	Petunidin 3-galactoside	38.3 - 40.4	Hosseinian *et al*., 2008 [26]
Red wheat	Malvidin-3-glucoside	0.06 - 0.43	Ficco *et al*., 2014 [16]
	Peonidin-3-galactoside	0.06 - 3.87	Ficco *et al*., 2014 [16] Abdel- Aal and Hucl, 2003 [1]
	Cyanidin-3-glucoside	0.59 - 10.23	Ficco *et al*., 2014 [16]

significant increase in total flavonoid content, total phenolic content, total anthocyanin content and antioxidant activity was observed in all colored wheat sprouts compared to the wheat grains. Among the colored wheat grains and sprouts total anthocyanin, quercetin and pelargonidin contents were highest in the sprouts of the purple wheat genotypes than in the blue or yellow wheat genotypes. Delphinidin was detected at a higher amount in the grains than in the sprouts of the blue wheat genotypes. Peonidin was detected at very low quantities in the grains of all colored wheat genotypes. Increased total anthocyanin content was found in the sprouts of the yellow wheat genotype while the lowest total anthocyanin content was observed in the grains of yellow wheat genotypes due to the high carotenoid and low anthocyanin level in the yellow wheat grains. The purple wheat sprouts had the highest pelargonidin, cyanidin and quercetin contents among all other colored genotypes and hence can be a promising source for functional food use.

Antioxidant activity of colored grains

Antioxidants are compounds having free-radical scavenging activity, transition-metal-chelating activity, and/or singlet-oxygen-quenching capacity. Antioxidant activities of colored grains are due to the presence of anthocyanins in them. Among these anthocyanins, cyanidin-3-glucoside possesses an antioxidant activity 3.5 times stronger than Trolox (vitamin E analogue). Measurement of antioxidant activity is mainly done using DPPH (2, 2-diphenyl1-picrylhydrazyl test), ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid method), FRAP (ferric reducing antioxidant power method) and ORAC (oxygen radical absorbance capacity) assays.

Yao *et al*., 2010 [74] conducted an antioxidant analysis in red, purple and black rice, purple corn and black barley samples. For the study total phenolic content (TPC) was measured using a Folin-Ciocalteu method, total antioxidant activity was determined by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity. According to the results among the studied colored grains black rice had the highest TPC that was 86 times greater than that of red rice. Also among the cereals black rice possess the highest total anthocyanin content (TAC) followed by purple corn. In red rice the concentration of anthocyanin was very low because only a small cyanidin-3-glucoside peak was detected during the present conditions of study. In case DPPH free-radical-scavenging capacity black rice had the highest value (73.47 µM TE/g) and red rice had the least DPPH free-radical-scavenging capacity value (1.68 µM TE/g).

Rice

Sompong *et al*., 2010 [63] conducted a study on nine red and three black rice varieties from Thailand, China and Sri Lanka and evaluated the proximate composition, physicochemical and antioxidant activities. In case of total phenolic content (TPC) it differed significantly between the varieties but not between the colors and highest TPC was observed in the red Thai rice Bahng Gawk (BG) (691 FA equivalent mg/100 g dry matters) and it also exhibited highest antioxidant activities. It was found that antioxidant capacity did not differ significantly between red and black rice varieties but varied amongst genotypes. According to FRAP antioxidant capacity of rice varieties ranged between 0.9–8.1 mmol Fe (II)/100g dry matter and for trolox equivalent antioxidant capacity assay it was between 2.1–12.3 mmol TEAC/100g dry matter. DPPH Scavenging ability was in the order Thai rice > Chinese rice > Sri Lankan rice, where the highest ability to scavenge DPPH radical was found for Bahng Gawk i.e. a red Thai rice variety (13.0% remaining DPPH). In case of Sutharut *et al*., 2012 [66] study was focused on the effect of germination on total anthocyanin content and antioxidant activity of black colored rice. For the study normal, non-waxy rice variety Phitsanulok 2 was used as control and two pigmented rice including black glutinous rice locally named Niew Dam and black non-waxy rice locally named Hom Nil were used. For the research rough rice and dehulled rice kernels were kept in water for 12 and 6 hours, respectively before further left to germinate for 0, 6, 12, 18, and 24 hours and the result showed that rice grains without husk showed higher germination rate compared to those of rough rice. Total anthocyanin content (TAC), trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP) of the germinated black rice samples were evaluated and the results indicated that TAC and TEAC of black glutinous rice were higher than that of the other two samples. Also black rice germinated from rough rice had higher TAC content and TEAC values than that of grains germinated without husk. So it can be concluded that rice with husk intact should be employed for the production of germinated pigmented rice to reduce the TAC loss during germination process.

Peanparkdeea *et al*., 2019 [49] studied the bran portion of two Thai non-glutinous rice called Khao Dawk Mali 105 (white rice), hom nil (purple rice) and two Thai glutinous rice called kiaw ngu (white rice), leum Pua (dark purple rice) to find their compositions, phenolic content and antioxidant activity. As per the results 65% (v/v) ethanol had good polarity to extract phenolic acids, flavonoids, and anthocyanins from the rice brans compared to other solvents such as isopropanol and isopropanol/hexane (1:1) while higher amounts of γ-oryzanol were found in isopropanolic bran extracts. The high phenolic acids, flavonoids, anthocyanins, total phenolic content, and antioxidant activity based on DPPH radical scavenging activity and FRAP values for all the cultivars were observed in bran extracts obtained by 65% (v/v) ethanol. According to the results obtained the purple glutinous variety leum pa had highest FRAP values (855.59 mmol Trolox/g) compared to other rice varieties at extraction temperature 60˚c. The FRAP assay results were in descending order, leum Pua > hom nil > kiaw ngu > khao dawk mali 105 at 60˚c. In case of DPPH activity highest value was observed in hom nil rice variety (17.69 mmol Trolox/g) at extraction temperature of 60˚c. The DPPH values were in descending order, hom nil > leum Pua > kiaw ngu > khao dawk mali 105 at 60˚c. Also it was found that TPC in rice bran extracts reduced as the time of extraction reached 90 minute and there was no significant difference between the FRAP values at extraction times of 60 and 90 min. This indicates that prolonging extraction time could not increase the TPC and antioxidant activity of rice bran extracts and the ultrasonic treatment at high temperature for a longer duration may cause chemical decomposition of phenolics, flavonoids, and anthocyanins in the colored rice bran extract.

Maize

Ku *et al*., 2014 [33] measured the total carotenoids, phenols, flavonoids, and anthocyanins from 40 Korean colored corn genotypes to analyze the correlation between antioxidant activity and these phytochemicals. The phytochemicals were extracted using methanolic HCL and FRAP, ABTS activity were measured for the antioxidant analysis as well. According to the results there was a large coefficient of variation (CV) in total anthocyanin (CV 85.0%) and total carotenoid contents (CV 87.8%) and in case of total phenol, total flavonoid contents, ABTS and FRAP the CV was relatively low (CV 15.0%, 22.8%, 15.5%, and 16.3% respectively). Correlation was done using pearson’s correlation analysis and there were meaningful correlations between ABTS and anthocyanins, phenols, and flavonoids, as well as correlations between FRAP, phenols and flavonoids. Anthocyanins from Korean colored corn correlated more with ABTS assay and flavonoids from Korean colored corn correlated with FRAP assay. Anthocyanins and carotenoids indicated large variation compared to other compounds and mainly anthocyanin had higher variations and strong correlation to antioxidant activity while flavonoids and phenols had fewer variations and carotenoids had less correlation with antioxidant activity. Thus anthocyanin can be considered as a good target to increase antioxidant activity in colored corns. A more informative and easily visualized result was obtained using principal component analysis (PCA).

Lopez-Martinez *et al*., 2008 [39] analyzed free-radical scavenging activity and phytochemical content (total phenolic, anthocyanin and ferulic acid) in eighteen Mexican maize phenotypes. Total phenolic contents of the phenotypes were between 215.8 to 3400.1 mg gallic acid/100 g of whole grain flour and total anthocyanin content of the phenotypes were between 1.54 to 850.9 mg cyanidin-glucoside equivalents/100 g of whole grain flour. The phenolics in grain were in the bound form while anthocyanins were the major free phenolic compounds present. According to the results bound phenolic extracts of colored corn had higher anti-radical and reducing activities than free phenolic extracts from the same grain samples when tested at a normalized phenolic concentration. The phenotypes Veracruz 42 and AREQ516540TL showed greatest activities and these were purple-colored strains rich in anthocyanins. Also extracts from a red-colored phenotype Pinto also exhibited anti-radical activities. The differences in free-radical scavenging and reducing activities are dependent on the unique profile of anthocyanins and other phenolic compounds in each phenotype.

Barley

In this study Abdel-Aal *et al*., 2012 [4] evaluated the composition of free and bound phenolic acids and DPPH activity in 28 black, blue, and yellow barley varieties. For the study free phenolics were extracted using aqueous methanol solvent while bound phenolics were extracted following alkaline hydrolysis. According to the study total phenolic content and individual phenolic acids were highly correlated with free radical scavenging capacity of barley samples. It was observed that black and blue barley was related and distinct from yellow colored barley. In case blue colored barley samples there were insignificant differences in DPPH depletion percentage by free phenolics. However due to black and yellow colored barley significant differences were found i.e. blue barley samples showed a small range (57– 63%) on comparison with the black barley varieties (59–78%) and yellow barley varieties (53–79%) and for the free phenolic extract from the mixed-colored barley samples a high DPPH radical scavenging capacity (76%) was observed. In case of bound phenolic extracts the DPPH scavenging capacities were 61–79, 55–71, and 55–74% for the blue, black and yellow colored barley samples, respectively. These results indicate that barley genotype and color influence antioxidant capacity and this should be taken into consideration for the development of high-antioxidant barleys varieties.

Lee *et al*., 2013 [35] evaluated antioxidant activity of anthocyanin rich extract from hulless pigmented barley varieties. Extraction of anthocyanin was done using acetone and purification of the extract was conducted by solid-phase (C18) extraction cartridge. Purple barley cultivar had 11 anthocyanins and only one anthocyanin i.e. peonidin derivative was found in blue, black and yellow barley. TAC in colored barley genotypes was between 3.2 to 678.5 mg/kg in whole grain and from 4.5 to 1654.6 mg/kg in the bran portion. After the study it was found that purple barley bran extract had the highest DPPH radical scavenging capacity, superoxide radical scavenging capacity and total antioxidant activity. The DPPH radical scavenging capacity of 86.8% was observed in purple barley at anthocyanin concentration of 330.9 mL^{-1} and was comparable to 0.1 mg mL^{-1} ascorbic acid that showed 91% of the capacity. The superoxide radical scavenging capacity of colored barley had similar trend like DPPH radical scavenging capacity i.e. the superoxide radical scavenging capacity of purple barley with 330.9 mL^{-1} concentration of anthocyanin extract was equivalent to 90% capacity of 0.1 mg mL^{-1} ascorbic acid. The total antioxidant activity (TAA) of the extract was again highest in purple barley followed by blue and black yellow barley cultivars. TAA of purple, blue, black and yellow colored barley was equivalent to 79.3, 36.8, 29.3 and 12.3% antioxidant activities of 0.1 mg mL^{-1} ascorbic acid.

Wheat

Liu *et al*., 2010 [38] evaluated the antioxidant capacities against oxygen radical and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical of extract from six wheat varieties i.e. three purple, one yellow, two red, and one white. HPLC and mass spectroscopy was used to evaluate the phenolic composition in the colored varieties. According to the results charcoal purple wheat showed the highest ORAC value that is up to 6898 µmol/100g in the methanol extract and 1920 µmol/100 g in the acetone extract followed by Red Fife wheat, yellow Luteus wheat and white AC Vista wheat that had the lowest antioxidant activity due to its low phenolic content. Acidic methanol extract exhibited higher DPPH activity than acetone extract and for three purple wheat varieties the average value was 773 µmol/100g followed by yellow wheat (669 µmol/100 g) due to higher flavonoid content (13.44 mg/100 g) and then red wheat (648 µmol/100 g). The least DPPH radical scavenging activity was in the acetone extract of white wheat, AC Vista (423 µmol/100 g).The main phenolic compounds identified in colored wheat are phenolic acids, flavones, flavonols, and anthocyanins. Phenolic acids, flavones and flavonols were detected in all of the wheat varieties while anthocyanins were identified only in purple colored wheat variety.

Mrkvicová *et al*., 2017 [43] studied the antioxidant activity of purple Konini wheat having total anthocyanin content of 41.70 mg/kg in rats (32), chickens (32) and fishes (20). The same numbers of animals were fed with common wheat with TAC of 24.95 mg/kg as controls for the study. At the end of the study animals were killed and blood and liver tissues were taken for biochemical analyses. The antioxidant activity in the liver tissues was measured using the DPPH, FR (Free Radicals method), FRAP and ABTS methods. In case of rat fed with konini diet DPPH, FR, ABTS values were 10.06, 22.99 and 19.67 mg GAE /g of protein respectively and the DPPH and FR values different from control while ABTS value was similar to control. The chickens fed with Konini wheat showed significantly different values from control using DPPH (10.01 mg GAE/g of protein), FR (26.13 mg GAE/g of protein) and ABTS (21.01 mg GAE/g of protein) methods. In case of fish the value of DPPH, FR and ABTS were 14.91, 22.97 and 14.74 mg GAE/ g of protein respectively but there wasn't much difference from the control. The result indicates that feeding a higher content of anthocyanins can improve the antioxidant activity.

Health benefits of colored grains

Colored cereals are found to have the presence of anthocyanins and these compounds have been known as health-promoting functional food ingredients due to their antioxidant activity (Nam *et al*., 2006; Philpott, 2006) [44, 51], anticancer (Hyun & Chang, 2004; Zhao *et al*., 2004) [28, 78], hypoglycemic (Tsuda *et al*., 2003) [72], anti-inflammatory effects (Tsuda *et al*., 2002) [71] etc.

Ling *et al*., 2001 [37] evaluated the effect of black, red and white rice consumption on hypercholesterolemia induced atherosclerotic plaque formation in rabbits. For the study purpose 36 male rabbits were divided in to groups and were fed a normal laboratory purified diet (normal group), a high cholesterol diet (HC group), a high cholesterol diet with 30 g/100 g white rice (WR group), 30 g/100 g red rice (RR group) or 30 g/100 g black rice (BR group) for 10 week period. During the study blood samples were collected for lipid measurements and aortas were removed for evaluation of atherosclerotic plaques at the end of the procedure. Also the oxidant and antioxidant conditions of blood, erythrocytes, liver and aorta were evaluated during the study. It was found that in case of rabbits fed with red or black rice diets the area of atherosclerotic plaque was 50% lower than those fed with white rice diet. Also on comparison group fed with red rice and black rice diet had higher serum HDL cholesterol and apolipoprotein (apo) A-I concentration than group fed with high cholesterol and white rice diet. Similarly liver reactive oxygen species (ROS) and aortic malondialdehyde (MDA) were significantly lower and the liver total antioxidative capacity (TAC) and erythrocyte superoxide dismutase (SOD) activity were significantly higher in the group fed with red rice and black rice diet compared with high cholesterol and white rice diet. This indicates that the consumption of red or black rice reduced or retarded the atherosclerotic plaque development induced due to dietary cholesterol. Also enhanced serum HDL cholesterol, apo A-I concentrations, increased antioxidant and decreased oxidative status can be the mechanisms of the antiatherogenic effect of red or black rice.

Guzmán-Gerónimo *et al*., 2017 [22] evaluated the effect of blue maize extract on metabolic syndrome (MS) of wistar rats. Metabolic syndrome has a range of medical conditions such as elevated blood pressure, high serum triglycerides and low high-density lipoprotein (HD) levels. In this study wistar rats were fed with a diet high in sucrose and cholesterol for a period of 4 weeks and the effect of blue maize extract on metabolic syndrome was studied. It was found that the intake of blue maize extract increased the level of HDL from 1 to 1.2 mmol/L, decreased the levels of serum triglycerides from 2.5 to 1.7 mmol/L, decreased total cholesterol from 2.2 to 1.7 mmol/L, decreased systolic blood pressure from 162 to 112 and decreased epididymal adipose tissue weight from 10.5 to 8.1g in the rats with metabolic syndrome. It should be taken in to consideration that metabolic syndrome relieving effect may not only be due to the anthocyanins (2.9 mg/kg body weight) but also due to the other polyphenols since the extract contained 70% of the later one (total polyphenol content at 9.97 mg/kg body weight).

Zhang *et al*., 2021 [77] conducted a study on purified anthocyanin extract (PAE) from purple highland barley bran (PHBB) for antioxidant and neuroprotective effect. Liquid chromatography-mass spectrometry (LC-MS) analysis of PHBB from the Tibet Plateau in China showed a complex and highly acylated anthocyanin profile. PAE resulted in high antioxidant and neuroprotective effects on cobalt chloride ($CoCl_2$)- induced hypoxic damage in PC12 cells by sustaining the cell viability, restoring cell morphology, reducing lactic dehydrogenase (LDH) leakage, reducing reactive oxygen species (ROS) levels, increasing antioxidant enzyme activities, inhibiting cell apoptosis, and attenuating cell cycle arrest. Treatment cells i.e. PC12 and U2OS with PAE activated autophagy shows that autophagy possibly acted as a survival method against $CoCl_2$-induced injury. According to this study PAE from the PHBB can be potentially used as a preventive agent for brain dysfunction caused by hypoxic damage.

Chen *et al*., 2013 [12] conducted a study in order to find the antiaging and antioxidant properties of purple wheat on nematode *Caenorhabditis elegans*. For the study young adult worms 3 days after hatching were grown and kept for the whole life span in S-basal medium with living E. coli OP50 also containing purple wheat methanolic extract. According to the research it was clear that purple wheat methanolic extract rich in anthocyanin extended the mean life span of wild type worms and of mev-1(hn1) mutants that are sensitive to oxidative stress by 10.5 and 9.2 percentages, respectively and no significant increase of life span was observed in daf-16 (mgDf50) mutant worms. Also the extension of life span depends on the transcription factor DAF-16. Translocation of DAF-16/FOXO to the nucleus implies that the transcription factor DAF-16/FOXO was activated due to purple wheat treatment due to the inhibition of the insulin/IGF-1-like signaling pathway which includes the insulin receptor DAF-2. Also purple wheat extract increased stress response in *C. elegans* and reduced oxidative stress. Since anthocyanins of purple wheat extract exhibit beneficial effects in *C. elegans* they may exhibit similar properties in humans and this should be considered for further studies.

Application of colored grains in food industry

During these years many researches have been conducted on the development of functional foods using colored cereals and the main area of interest is baked and extruded products. Mainly bread, biscuit, pasta, noodles are the products being studied for the food application of colored cereals and the production is only in small scale and this should be done in large scale due to nutritional and health promoting properties of these cereals.

Thao *et al*., 2018 [69] studied the effect of pH on bioactive compounds (total phenolic content, anthocyanin content and gamma-aminobutyric acid content) and antioxidant activity of germinated colored rice and also evaluated the effect of adding different ratios (0, 10, 0, 30, 40 and 50%) of germinated colored rice in fresh rice noodles. Riceberry (RB) and Hom-Nil (HN) species of colored rice were germinated evaluated for the effect of pH on bioactive compounds and antioxidant activity. According to the results TPC, TAC, gamma-aminobutyric acid (GABA) content and antioxidant activity i.e. DPPH and FRAP assay were higher when the colored rice varieties were soaked in water at a pH 6 compared to pH 7. In case of RB variety at pH 6 TPC, TAC, GABA, FRAP and DPPH values were 4.18 mg GAE/ g, 0.25 mg C3G/g, 5.36 mg/g, 54.51 μmol FE (II)/g, 16.55 μmol TE/g respectively. In case of HN variety at pH 6 TPC, TAC, GABA, FRAP and DPPH values were 3.41 mg GAE/ g, 0.13 mg C3G/g, 6.06 mg/g, 45.32 μmol FE (II)/g, 10.79 μmol TE/g respectively. When the addition of germinated colored rice varieties increased, proximate composition and bioactive compounds in fresh noodles were also increased. In case of textural profile analysis of fresh noodles there was a significant decrease in terms of cohesiveness, springiness and chewiness due to the addition of 50% germinated colored rice. When the addition of germinated colored rice increased the lightness of the noodles decreased and redness and yellowness increased. According to the result of sensory analysis conducted by 30 untrained panelists there was no distinct difference in the noodles by the addition of 10 and 20% germinated colored rice. This can be concluded that the addition of germinated colored rice up to 20% can be used in fresh rice noodles.

Hernández-Uribe *et al*., 2010 [25] conducted a study on fresh and stored maize tortillas made from white and blue maize cultivars for physicochemical, rheological and structural characteristics analyzed by calorimetry, x-ray diffraction, Fourier transform infrared (FTIR) spectroscopy, dynamic viscoelastic tests, and high-performance size exclusion chromatography. Retrograded amylopectin was the reason behind the low temperature endotherm (50–56 °C) and retrograded amylose was the reason behind the high temperature endotherm (105– 123 °C). In case of fresh tortillas it showed an amorphous starch arrangement by x-ray diffraction study while in case of stored samples presence of peaks at $2\theta = 17^{\circ}$ and 23° indicates the re-crystallization of starch components. According to FTIR results development of higher levels of starch crystals were found during storage also blue tortillas had a lower absorbance ratio compared to white tortillas, indicating that the blue tortilla has lower tendency toward starch retrogradation. There was a difference in the viscoelastic parameters of fresh and stored samples i.e. at the longest storage times; white tortillas were more rigid compared to blue tortillas. In case of molar mass values for starch it increased for both white and blue tortillas as storage time progressed and relatively higher values were obtained for white tortillas on comparison with blue tortilla. According to the study starch reorganization occurred more in white tortillas by calorimetric, x-ray diffraction, FTIR and rheological methods on comparison with blue one and the changes occurring in tortillas during storage are related to reorganization of starch components and here the maize variety is of more importance than the color.

Martínez-Subirà *et al*., 2020 [40] conducted a study on biscuits prepared from purple barley with higher bioactive compounds. For the study β-glucans, arabinoxylans, anthocyanins and other phenolic components of biscuits containing different proportions of whole purple barley flour and pearling fractions were evaluated and these were compared with biscuits made from 100% refined wheat flour and 100% whole wheat flour. Result showed higher antioxidant capacity and lower glycemic index GI in purple barley based biscuit than refined wheat and whole-wheat biscuits. The physical characteristics of the biscuits were little worse than the refined wheat based biscuits but similar to whole wheat biscuits. Baking process did not affect β-glucans and arabinoxylans content that favored the release of phenolic compounds from the food matrix and hence increased antioxidant capacity. The anthocyanins from purple barley were thermally unstable and decreased after baking and this was improved by addition of tartaric acid. The biscuit made from 100% purple barley provides more than 0.75g of β-glucans and hence one serving of four biscuits satisfy the 3g of β-glucans requirement per day and this will help to reduces blood cholesterol and risk of heart diseases.

Pasqualone *et al*., 2015 [48] conducted a research anthocyanin-rich biscuits from purple wheat and control biscuits were produced from a non-pigmented wheat cultivar for comparison. It was found that purple biscuits had a high level of total anthocyanins expressed as 13.86 mg/kg cyanidin-3-glucoside on comparison with non- pigmented wheat. In case of total phenolic content the purple wheat biscuit (2.58 mg/g ferulic acid equivalent) had higher value compared to non-pigmented wheat cultivar (1.37mg/g ferulic acid equivalent). Antioxidant activity (AA) was measure by DPPH and the result indicated that higher AA was observed in purple wheat biscuit (5.32 μmol/g trolox) compared to non- pigmented one (3.01 μmol/g trolox). According to the volatile compounds profile of purple biscuits it indicated lower levels of lipid-derived carboxylic acids and higher levels of alcohols and aldehydes than in the control biscuits and this shows a lower oxidative degradation of the lipid fraction in them. In case of sensory analysis the biscuits showed significant differences in color and friability, whereas no significant differences were observed in taste attributes (sour, salt, sweet and bitter taste).

Effect of processing on bioactive compounds in colored grains

Amount of anthocyanin in colored grain is greatly affected by the processing conditions and hence it is necessary to check the stability of this pigment in different food products in order to have a successful food application of the colored grains. According to Sivamaruthi *et al*., 2018 [62] pH, temperature, enzymes, light and storage are the factors affecting the stability of anthocyanin.

Walter *et al*., 2011 [73] studied about the concentration of total soluble phenolic compounds (TSPCs) and antioxidant activity (AOA) of light brown, red and black rice grains and the processing effect on concentration of TSPCs in these pigmented grains. Parboiling process reduced the TSPCs concentration in the grains due to the loss of part of them in the processing water, thermal decomposition and possibly by the interaction with other components. For parboiled unpolished grains a reduction in the concentration of TSPCs was observed compared to raw unpolished rice i.e. a reduction of 48.6% for light brown pericarp color, 73.0% - 87.0% for red pericarp color grains and 32.8% for black pericarp color grains was observed. Phenolic compounds between 10.77 mg GAE and 39.24 mg GAE were found in the parboiling water and this shows that a small part of the reduction in the polyphenol content in the parboiled grains is due to the loss of polyphenols in the parboiled water. Parboiling process results in reorganization of the internal structure of the grains using starch and proteins. Polyphenols are affected by the formation of these complexes with proteins and thus becomes unavailable. Hence a reduction in polyphenol concentration in the parboiled grains was observed since the soluble phenolic compounds are quantified in this work. This reduction is related to the lower AOA level in these grains thus a significant correlation is detected between the concentration of TSPCs and AOA in the grains. Thus irrespective of the type of processing AOA of the extracts obtained from rice grains is related to the presence of phenolic compounds. On comparison of parboiled polished grains with polished grains no significant difference was found in the concentration of TSPCs for most of the genotypes because polyphenols in the rice are mostly found in the external layers of the grain and are removed during polishing process and a small quantity of these compounds are left in the polished grain, this explains the reason for no difference between parboiled polished grain and polished grain. On comparison of TSPCs concentration in raw grains and cooked unpolished grains a reduction between 20.9% - 72.0% was observed and in case of cooked polished grains a reduction between 39.6% - 62.2% was found. In parboiled grains the effect of cooking was smaller i.e. a reduction between 12.0% - 32.6% was observed in cooked parboiled unpolished grains and between 15.1% - 27.8% was observed for cooked parboiled polished grains. This reduction in polyphenolic content after cooking is related to thermal decomposition since phenolic compounds are affected by high temperatures treatments.

De la Parra *et al*., 2007 [13] evaluated the phytochemical profile (total phenolic content, anthocyanins, ferulic acid and carotenoids) and antioxidant activities of five types of corns such as white, yellow, high carotenoid, blue and red. These pigmented corn varieties were processed into masa, tortillas and tortilla chips and studied for their phytochemical and antioxidant activity. Among the raw corns highest total phenolic content, ferulic acid content and antioxidant activity were observed in the high-carotenoid genotype (phenolic content: 320.1mg GAE/100g dm, ferulic acid content: 153012 µg ferulic acid /100g dm) followed by the yellow corn (phenolic content: 285.8 mg GAE/100g dm, ferulic acid content: 102968 µg ferulic acid /100g dm). White corn exhibited lowest total phenolics contrent (260.7mg GAE/100g dm) and antioxidant activity. In case of anthocyanin content blue corn (36.87 mg cyanidin-3-glycoside equivalent /100 g dm) had the highest anthocyanin concentration and yellow corn had the lowest anthocyanin content (0.57 mg cyanidin-3-glycoside equivalent /100 g dm). According to the study, products prepared through nixtamalization had reduced total phenolic content and antioxidant activities on comparison with raw corns. In case of free phenolics and soluble conjugated ferulic acid nixtamalized corn products exhibited higher amount and had lower amount of bound phenolics and ferulic acid than raw corns. Among the products made from these corns high carotenoid corn tortillas had highest phenolic content (207.5 mg GAE/ 100g dm) and ferulic acid content (136616 µg ferulic acid /100g dm). Lowest phenolic content was found in red corn chips (111.7 mg GAE/ 100g dm) and lowest ferulic acid content was found in white corn masa (42229 µg ferulic acid /100g dm). In case of anthocyanin content of the products made from these corns highest value was found in blue maize tortilla (3.81 mg cyanidin-3-glycoside equivalent /100 g dm) and lowest was found in white corn masa (0.28 mg cyanidin-3-glycoside equivalent /100 g dm). The study suggests that lime-cooking had resulted in significant reduction in the phytochemical content of nixtamalized products but enhanced release of phenolics and ferulic acid.

Bartl *et al*., 2015 [9] evaluated difference in anthocyanin content between breads made from the blue and purple wheat grains at different baking temperatures and times i.e. at 240˚C for 21 min and 180˚C for 31 min. After the evaluation, bread prepared at 240˚C from blue wheat whole meal flour had 8.33 mg/kg anthocyanins (decrease of 7.1%) and bread from purple wheat had 5.2 mg/kg anthocyanins (decrease of 61%). In case of bread prepared from blue wheat at 180˚C had 5.31 mg/kg anthocyanins (decrease of 40.8%) and purple-grained wheat had 3.63 mg/kg anthocyanins (decrease of 72.8%). This indicates that loss of anthocyanin depended on the baking procedure and was highest after baking at 180 ˚C for 31 minute than baking at 240˚C for 21 minute. The loss of anthocyanins was highest in bread baked from purple wheat than blue wheat bread and this suggests that acetylated anthocyanins in purple wheat but not in blue wheat have more chances of thermal degradation during baking.

Conclusion

This review focuses on bioactive compounds, antioxidant activities, health benefits, application and effects of processing on colored cereals like rice, maize, barley and wheat. It was found that most studies were related to phytochemical composition and antioxidant activities of colored cereals and also the total phenolic content and total anthocyanin content is directly related to antioxidant activity of the pigmented grains in many of the studies. The process of germination of colored grains improves their nutritional and antioxidant properties and this area should be taken more in to consideration for further studies. Health benefit related studies were conducted in animal models, clinical and human trials are needed to be done in order to confirm these properties and also bioavailability of these anthocyanins from pigmented cereals in humans should also be taken in to consideration for future studies. Applications of colored cereals are found in extruded and baked products and more areas of application should be studied.

Declaration of competing interest

The authors declare that they have no known competing financial interests that could have appeared to influence the work reported in this paper.

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