Nutrients, Nutraceuticals and Bioactive Properties of Multi-Whole Grain Mix for Drink and Porridge

Hameeda Banu, N. Itagi, A. Jayadeep* and Vasudeva Singh

Department of Grain Science and Technology, Central Food Technological Research Institute (Council of Scientific and Industrial Research), Mysore – 570 020, Karnataka, India

Abstract: Whole grains are reported to be rich in nutrients, nutraceuticals and have number of health beneficial effects. A convenient multi-whole grain mix for the preparation of a drink or porridge was formulated by using cereals, millets, pulses and nuts. Particle size was mostly of 180-250 microns (52%). Amylograph characteristics like GT, PV, HPV, CPV were 82°C, 285BU, 310BU, and 605BU, respectively were ideal for drink. The mix was found to be rich in carbohydrate, protein, fibre and calorie. The 100g of the mix had nutraceuticals like carotenoids (290µg), γ-tocopherol (4.6mg), α-tocopherol (1.5mg), and polyphenols-soluble, bound and total (94,132 and 226mg GA Eq.). Bioactive properties like vitamin E activity, free radical scavenging activity, total antioxidant activity and starch digestibility were 2.6i.u., 153mg catechin.Eq./100g, 17mg Tocopherol equivalent and 61.8%. Mix was sensorily acceptable in the form of drink and porridge and can be used as an ideal nutritious food for all age group.

Keywords: Whole grain, viscosity, sensory, nutrient, nutraceutical, carotenoid, tocopherols, polyphenols, antioxidant, bioactivity.

INTRODUCTION

Whole grains contain all parts of the grain viz., the endosperm, germ, and bran. Whole grains are rich in nutrients and phytochemicals with known health benefits. As reviewed by [1], whole grains have high concentrations of dietary fibre, resistant starch, and oligosaccharides. They are also rich in antioxidants including trace minerals and phenolic compounds and these compounds have been linked to disease prevention. Other protective compounds in whole grains include phytate, phyto-oestrogens such as lignan, plant stanols and sterols, and vitamins and minerals. Epidemiological studies find that whole-grain intake is protective against cancer, CVD, diabetes, and obesity. Whole grain feeding studies in human subjects also report improvements in biomarkers such as weight loss, blood-lipid improvement and antioxidant protection.

The major cereals and millets consumed as whole grains in India are wheat, sorghum, Pearl millet, finger millet and to some extent brown rice. These grains are the major source of nutrients in diets contributing to around 7.3-11.6 % protein, 1.3-5 % fat, 60-72 % carbohydrate, and 328 - 361 kcal of the daily energy intake [2].

The foods which we commonly consume also contains tocopherols, carotenoids, phenolics and flavanoids, which serve as a good source of natural antioxidants [3,4,5] and are reported to have health beneficial effects [6]. Vitamin E is a fat-soluble vitamin and includes both tocopherols and tocotrienols [7]. It has many biological functions especially as antioxidant that stops the production of reactive oxygen species [8], neuroprotective [9], inhibition of platelet aggregation [10], control of gene expression etc [11].

Carotenoids are important in human nutrition and health. They are valuable as antioxidants [12], in the prevention of atherosclerosis in the maintenance of immune function [13], in the health of eyes [14] and some are precursors of vitamin A. Presence of carotenoids was reported in grains such as maize, wheat and sorghum [15, 16].

Cereals and legumes contain a wide range of phenolics and act as good source of natural antioxidants [17]. Presence of antioxidative phenolics have been reported in millets also [18, 19]. Flavonoids like tannin and anthocyanins also have antioxidative potential [20]. All these indicate the bioactive potential of whole grains. However, number of palatable products from individual whole grains and multiple whole grains are few. Reports on the nature of lipid soluble as well as other polyphenolic antioxidants of the multi-whole grain products are also limited.

Reports on multigrain weaning foods based on polished rice, malted legumes and millets are also reported [21, 22]. However, drink or porridge mixes from multi-whole grains are scanty. Hence a convenient multi-whole grain mix was formulated by

*Address corresponding to this author at the Department of Grain Science and Technology, Central Food Technological Research Institute (Council of Scientific and Industrial Research), Mysore – 570 020, Karnataka, India; Tel: +91 821 251 0843; Fax: +91 821 251 7233; E-mail: jayadeep@cftri.res.in

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selecting nutrients and nutraceuticals rich cereals, millets and other ingredients, and optimization of conditions for development of flavor and appropriate texture. Main objective was to assess the quality of the formulated product with respect to physico-chemical, viscographic parameters, nutraceuticals and bioactive properties, and also the sensory quality of the cooked product for use as drink or porridge.

MATERIALS AND METHODS

Materials

Cereals and millets – red rice (Oryza sativa), wheat (*Triticum aestivum*), maize (*Zea mays*), Pearl millet (*Pennisetum typhoideum*), Sorghum (*Sorghum vulgare*), barley (*Hordeum vulgare*), finger millet (*Eleusine coracana*), little millet (*Panicum sumatrens*); pulses – whole green gram (*Phaseolus aureus Roxb*), roasted bengal gram (*Cicer arietinum*); nuts - cashew nut (*Anacardium occidentale*), ground nut (*Arachis hypogaea*); sago and cardamom (*Elettaria cardamomum*) were purchased from local market in Mysore, Karnataka, India, from a single batch. The grains were cleaned, kept in air tight polyethylene bags, in a cool and dry place prior to use.

Gallic acid, catechin, tocopherol, β-carotene, pepsin, pancreatin, termamyl and amyloglucosidase were from M/s Sigma Chemical Co., (St. Louis, MO, USA). Tocovid capsule of Hovid Bhd, Malaysia used for tocotrienols. All other chemicals were of analytical grade.

Preparation of RTC Multi-Whole Grain Mix

Whole grain mix was prepared by roasting the grains individually, mixing in suitable proportion, pulverization and size reduction (Figure **1**).

Analysis of Particle Size Distribution

The particle size distribution of the multi-whole grain mix were studied by shaking 100 g of sample for 10 min in a set of Jayant Standard Test Sieves (Jayant Scientific Industries, Mumbai, India) ranging from mesh no. 28 to 100 or openings of 600 to 150 µm, in a sieve rack fitted in a Rotap sieve shaker. Sieved fractions were weighed.

Colour

Colour values of the RTC multi-whole grain mix was determined by using Hunter lab scan XE model (M/S Hunter associate laboratory Inc., Reston-V.A., USA) with a view angle of 2°. Colour values of the sample was determined by the Hunter system L, a, b values.

Bulk Density (BD), and Sedimentation Volume (SV)

The bulk density (BD) of the RTC multi-whole grain mix was determined according to the method of [23]. Sedimentation volume of the RTC multi-whole grain

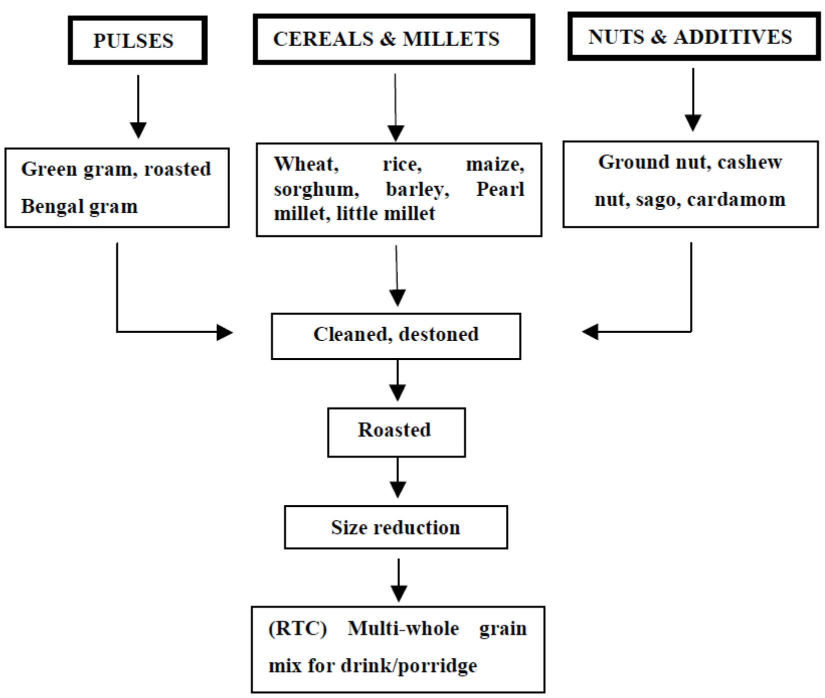

Figure 1: Flow chart for the preparation of RTC Multi-whole grain mix.

mix was determined according to the method of [24]. Two gram of fine multi-whole grain mix was taken in a 25 ml stoppered cylinder and 25 ml 0.05N HCl was added and the contents were shaken vigorously. Thereafter, 2 drops of amyl alcohol was added and the contents were shaken gently. The cylinder was placed undisturbed for about 4 h. Sedimentation volume was recorded at the end of 4h and percentage increase in volume was reported.

Pasting Characteristics and Apparent Viscosity

The pasting characteristics of multi-whole grain mix at 10% concentration was studied with a Brabender Viscograph, Type VSK 4 (Duisburg, FRG) fitted with a 700 cmg sensitivity cartridge using the procedure described in [25]. Viscosity of cooked multi-whole grain drink was measured at 10% concentration by using Brookfield DV – II + Pro (Brookfield Engineering Laboratories, INC, MA USA), at room temperature using disc spindle measuring system in a Synchro-electric viscometer. Apparent viscosity was measured as a relation between torque and spindle speed rotation. Measurement was carried out at 100 rpm using spindle number 2 depending on the measurement range of sample viscosity.

Proximate Analyses

The moisture content of the RTC multi-whole grain mix was determined by drying at 130° C for 2 h as per [26]. The micro Kjeldhal method was employed to determine the total nitrogen and the protein content (Nx6.25) [27]. Fat was estimated by extraction with petroleum ether (60 - 80° C), with a soxhlet apparatus and ash content was determined as per [27]. Carbohydrate was calculated by difference method as 100 – (moisture+protein+ash+fat). Dietary fibre was estimated using the rapid enzymatic assay of [28].

Determination of Total Carotenoids

Total carotenoid was determined by the procedure of [29]. Five gram of sample was mixed with about 50 ml of acetone and ground with pestle and mortar. The extract was filtered and the extraction repeated till sample becomes colourless. The extracts were pooled and mixed with 50 ml petroleum ether and 400 ml distilled water in a separating funnel. The petroleum ether layer was separated and washed 2-3 times with water, dried with anhydrous sodium sulphate and made up to 100 ml with petroleum ether. The absorbance was measured at 452 nm and the total carotene content was calculated based on the molar extinction co-efficient of β-carotene.

Characterization of Carotenoids by HPLC

The solvent used for extraction of total carotenoid was evaporated using nitrogen at 40-50° C in a water bath and the residue was dissolved immediately in a known volume of methanol and stored at -20° C until analysis. The carotenoids were fractionated on C18 (250 x 4.6 mm, 5 µm) column using HPLC system [Shimadzu HPLC with SCL-10A system controller, LC 10AT pump, and SPD, 10A UV- visible detector] and isocratic solvent system containing acetonitrile, chloroform, isopropanol and water (78: 16: 3.5: 2.5 v/v) set at a flow rate of 1 ml/min and the detector was set at 452 nm. Linear response of carotenoid was in the range 0.5-10 ng [30].

Extraction of Sample for the Vitamin E Analysis, Antioxidant Property and Polyphenol Analysis

The multi-whole grain mix (0.4 g) was extracted with 4ml of methanol for one hour with occasional stirring. The extract centrifuged at 3000 rpm and the supernatant was filtered, and then stored at -20°C until used for the analysis of Vitamin E, antioxidant property and soluble polyphenols [31]. Further for extracting the bound polyphenols, the residue was extracted and supernatant collected as above with 1% HCl methanol reagent [32].

Estimation of Polyphenols

Known quantity of methanolic extract of sample was mixed with Folin Ciocalteus reagent and sodium carbonate, volume adjusted with water, kept in dark for 30min, centrifuged and supernatant read for OD at 760 nm. Ferulic acid was used as standard [33].

Characterization of Vitamin E by HPLC

Vitamin E (tocopherols and tocotrienols) content of multi-whole grain mix was quantified by the method of [30], as explained by [5]. Reverse phase HPLC (CBM-10A Shimadzu system with RF10AXL fluorescent detector, LC10AT pump) was used and the chromatograms were recorded and processed by LC-10A class software. The extracts were separated on Merck Purospher Star C18 column (4.6X250 mm, 5 mm); (Merck, Darmstadt, Germany) using a gradient solvent system consisting of acetonitrile, methanol, isopropanol and aqueous acetic acid [45:40:5:10] as solvent A and acetonitrile, methanol and isopropanol

(25:70:5) as solvent B. The Fluorescence detector was set at excitation and emission wavelengths of 298 and 328 nm, respectively. Standards of both tocopherol and tocotrienol exhibited a linear response in the range as follows; α 4-45 ng, γ 3-55 ng, δ 0.4-5ng.

In-Vitro Starch Digestibility

In – vitro starch digestibility of multi-whole grain mix was estimated according to the method described by [34]. The glucose in the digested sample was estimated by DNS method [35].

Total Antioxidant Activity

The total antioxidant activities of the multi-whole grain mix was quantified using the phosphomolybdenum reagent [36]. An aliquot of methanolic extract of sample (20 µL) mixed with 1230 µl of the reagent in a microtube. The tubes were capped, shaken well and incubated at 90°C for 90 min in water bath and the absorbance was measured at 695 ηm against a reagent blank. Results were calculated and expressed as α-tocopherol equivalents (TE) per gram using the molar extinction coefficient of α-tocopherol. Linearity of reaction was found to be in the range 2×10^{-4} to 2×10^{-5} moles.

Free Radical Scavenging Activity

Methanol soluble extract of the sample was mixed with DPPH reagent, kept in dark for 30 min at room temperature and read for OD at 517nm. DPPH reagent was used as blank and percentage reduction was noted. Catechin was used as the standard and the catechin equivalent was calculated from the linearity curve of percentage reduction against the quantity [37].

Preparation of Drink/Porridge from the Ready to Cook Multi-Whole Grain Mix

Drink was prepared by mixing 10 g sample and 25 gm in the case of porridge, in a pan with 100 ml of water, 60 ml of milk, 2.5 g sugar. This slurry was cooked for 3-4 min in a thick vessel in low flame with continuous stirring. Cooking time was fixed based on sensory acceptability.

Sensory Evaluation

A trained panel was employed for carrying out sensory evaluation of multi-whole grain drink/porridge prepared from the mix were analysed for sensory quality acceptance by following the method of quantitative descriptive analysis (QDA). The scorecard consisted of 15 cm scale where in 1.25 cm was anchored as ‘Low’ and 13.75 cm as ‘High’ [38]. The sensory attributes such as appearance of buff colour, consistency, toasted cereal aroma, pulsy aroma, milk like aroma, cardamom aroma, bland and sweet taste and overall acceptability were analysed. Evaluations were carried out in sensory booths under white fluorescent light, air conditioned at 20±2 °C with relative humidity 50±5 %. The drink was served to panelists in cups and porridge in porcelain plates coded with 3-digit random numbers to minimize bias.

Statistical Analysis

Results are presented as mean $\pm$ SD (standard deviation) of three independent determinations [39].

RESULTS AND DISCUSSION

Physical Properties of the Mix

Particle size varied from 600 microns to <150 microns (Table **1**). Coarse particles were very less 0.36 %. Mix contains mostly 180-250 micron size particles (52 %). This make the quality of the cooked product smoother with better mouth feel. Endosperm hardness, amount of pericarp remaining after decortication, flour particle size distribution, etc. in sorghum is reported to affect the quality of porridge. Isolated sorghum starch (<45 microns) was of equal or better quality and contained less soluble solids than to prepared from sorghum flours containing particles <250 microns or <425 microns [40].

In the Hunter colour measuring system L value indicating the brightness was found to be 68.5 for the mix. Lower ‘a’ value indicated that reddish tint is less in the mix even though red rice and fingel millet were used. ‘b’ value of 14.7 indicate the yellow tinge since the mix was prepared using maize, wheat and finger millet which are rich in carotenoids. Percentage change (~257 %) in sedimentation value for the mix shows the high degree of partial gelatinization of the starches present in various grains which has undergone the process of roasting and consequent higher water absorption capacity. Bulk density of the mix was 0.45 kg/L indicating the fluffy nature of the mix.

Viscographic Parameters

The various parameters measured in the Brabender Viscograph are shown in (Table **2**). The mix at 10 %

Table 1: Colour and Particle Size Distribution of Multi-Whole Grain Mix

Physical properties Colour		Mesh aperture (microns)	Overtails (%)
L	68.53 ± 0.31	600	0.36 ± 0.10
a	0.31± 0.06	500	0.95 ± 0.30
b	14.70 ± 0.12	353	7.14 ± 0.67
Delta E (Δ E)	26.27 ± 0.27	250	21.73 ± 1.95
Sedimentation volume (% increase)	256.8 ± 4.71	180	52.20 ± 1.62
Bulk density (kg/L)	0.45 ± 0.00	150	14.89 ± 4.84
		<150	2.73 ± 1.01

Values are mean ± standard deviation of three independent determinations.

Table 2: Amylograph Characteristics of Multi-Whole Grain Mix and Apparent Viscosity

Parameters	Multi-whole grain mix
Gelatinization temperature (°C)	82 ± 0.00
Peak viscosity (BU)	285 ± 5
Hot paste viscosity (BU)	310 ± 10
Cold paste viscosity (BU)	605 ± 35
Break down (BU)	-25 ± 5
Set back (BU)	320 ± 30
Total set back (BU)	295 ± 25
Apparent viscosity (cps)	141.60 ± 0.65

Values are mean ± standard deviation of three independent determinations.

concentration on cooking had gelatinization temperature (82°C), indicating that the granules in this mix loses their birefringence at an early temperature due to the pre– gelatinization occurring at the roasting step of the processing. Peak viscosity (PV) is the viscosity where the starch granules reach highest swelling while cooking. It was observed that the mix showed least peak viscosity of about 285 BU, indicating that the various grains in the mix had undergone high degree partial gelatinization, which means grains had achieved almost cooked nature, while processing. Hot paste viscosity (HPV) is the viscosity registered at the end of cooking. It was observed that in the mix the HPV was 310 BU, and it was an indication that the swollen granules did not change or did not break, indicating that the mix was behaving like cross linked starch, as cross linked starch will not break down while cooking [41]. Break down value was negative, further indicating the behavior of cross linked type starch. The cold paste viscosity (CPV) value was 605 B U. Correspondingly the set back was less indicating the fact that the mix had undergone lesser retrogradation. Total set back value was low; hence the quantity of linear molecules precipitation was less in the mix. Where as PV, HPV and CPV were 570, 410 and 980 BU, respectively, in finger millet flour which is traditionally used for drink or porridge purpose which will have thick slurry [42].

Viscosity analysis by digital brook field viscometer of the cooked mix at 10% concentration showed the least viscosity (141.6 cps) at 45 °C indicating the semi crystalline nature of the starch granules.

Nutritional Composition

Moisture content of the mix was 8 %. Carbohydrate, determined by difference was 71 %, as this mix contained maize, wheat, barley as major grains which are rich sources of carbohydrates. Protein content was 10.5 % due to the presence of pulses and nuts. Lipid content was high (8.6 %), this could be due to the presence of maize, ground nut and cashew nut which are rich sources of lipid. Ash content was 1.92 % and calorie of the mix was 402 kcal. Insoluble dietary fibre was high (11.7 %) because of the presence of seed coat of the grains as mix was prepared from whole grains. Soluble fibre was 1 % and total dietary fibre was high 12.7 % (Table **3**). Investigations on whole grains of rice suggest that content of total dietary fibre is only 4.96-8.8 % [43], where as that in polished rice is still lower at 0.22 to 1.25 % [44]. The use of whole wheat flour instead of refined flour significantly improved the nutritional profile of flour tortillas [45]. There are different functional foods developed in different countries like USA, Japan, EU from whole grains considering its nutritional potential [46]. Effect of replacement of whole wheat flour with multigrain blend, increased the protein, fat, dietary fibre and mineral contents of north Indian parotta, [47]. Multi-whole grain mix also contributes significantly to RDA of protein (22 %), fibre (51 %) and calorie (18 %). This indicates that multigrain composition is nutritionally superior to refined grains. Whole grain and refined wheat flours showed distinct metabolic profiles in rats due to difference in the content of health beneficial components [48]. Whole grains and dietary fibre continue to win honors in preventing various diseases [49].

Table 3: Proximate Composition of Multi-Whole Grain Mix

Components	Multi-whole grain mix
Moisture (%)	7.99 ± 0.05
Total carbohydrate (%)	70.98 ± 0.31
Protein (%) (Nx6.25)	10.48 ± 0.21
Fat (%)	8.63 ± 0.17
Ash (%)	1.92 ± 0.00
Calorie (kcal/100g)	402.28 ± 0.85
Insoluble dietary fiber (%)	11.70 ± 0.24
Soluble dietary fiber (%)	1.00 ± 0.14
Total dietary fiber (%)	12.70 ± 0.33

Values are mean ± standard deviation of three independent determinations.

Nutraceuticals in Multi-Whole Grain Mix

Total Carotenoids and its Charecterization

The total carotenoids content in the mix was high 290 µg/100g. This could be due to the presence of whole grains like maize, wheat and Finger mullet in the mix. This value was comparable with the carotenoid content of the proso millet (366 µg/100g) [5], but significantly higher than the carotenoids content of wheat 150-200 µg/100g, sorghum 180-230 µg/100g, finger millet 199 µg/100g and little millet 78 µg/100g [16,5]. Whole multi-grain products will provide the good proportion of carotenoids. However, the presence of ß-carotene could not be detected by HPLC analysis (Figure **3**), unlike in maize which is reported to contain about 15.7 µg/100g [50]. Even then, contribution of other carotenoid types like xanthophylls (lutein and astaxanthin) detected in the mix should not be overlooked. These are present in a wide variety of fruits and vegetables and also in grains [51, 49]. Lutein and zeaxanthin are repoted to prevent eye diseases also [52].

Vitamin E Characterization

The characterization of vitamin E in multi-whole grain mix was carried out by reverse phase HPLC (Figure **4**). Vitamin E in the mix was found to be in the form of tocopherols as the major component and tocotrienols as minor component. The content of total tocopherols was 6.1 mg/100g. Gama tocopherol in the mix was higher (4.6 mg/100g) followed by alpha (1.5 mg/100g). Tocotrienols are not quantified since the contents are only in traces.

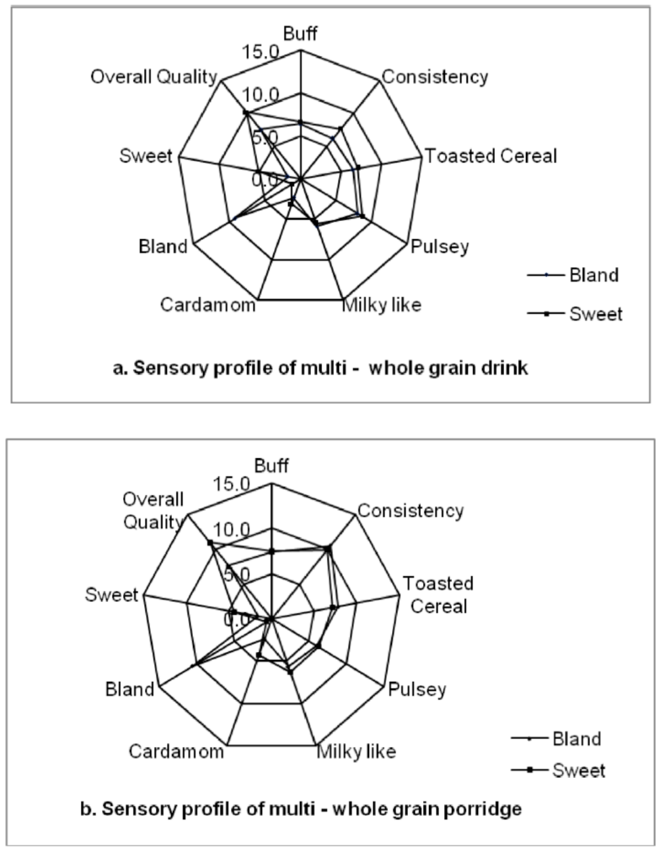

Figure 2: Sensory profile of multi-whole grain drink and porridge.

Vitamin E component in other cereals like wheat also is tocopherol where as the major component in rice is tocotrienols [53]. Rice and wheat flours contain only 0.8 mg and 1.23 mg/100g total Vitamin E, respectively, whereas the vitamin E content in refined little millet is 1.3 mg and whole grain finger millet flour is 4.1 mg/100g [5].

Vitamin E mainly exists as alpha, gamma and delta isomers. Among those, α-Tocopherol is an important lipid-soluble antioxidant and it protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction [54, 8]. Other forms of tocopherols like gama also have antioxidant properties and their own unique properties [7]. Both tocopherols and tocotrienols have antioxidant potential against cholesterol oxidation [55]. Since vitamin E has a number of health beneficial effects the multi-whole

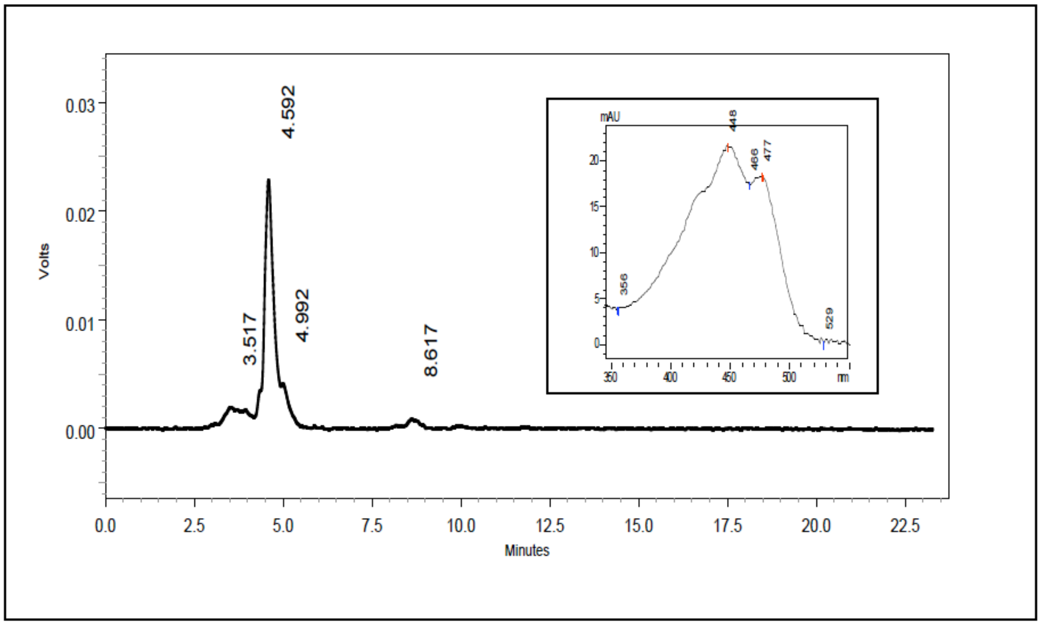

Figure 3: HPLC chromatogram of carotenoids in the multi-whole grain mix showing astaxanthin (3.5min) and spectrum of lutein peak (4.5 min).

grain mix could serve as a good source of these to the consumers.

Table 4: Nutraceuticals Content of Multi-Whole Grain Mix

Nutraceuticals	Multi-whole grain mix
Soluble polyphenols (mg GA Eq./100g)	94 ± 9.9
Insoluble polyphenols (mg GA Eq./100g)	132 ± 12.2
Total polyphenols (mg GA Eq./100g)	226 ± 23.3
Gama-tocopherols (mg/100g)	4.6 ± 0.3
Alpha-tocopherol (mg/100g)	1.5 ± 0.1
Total Tocopherols (mg/100g)	6.1 ± 0.7
Total Carotenoids (µg/100 g)	290.5 ± 0.5

Values are mean ± standard deviation of three independent determinations.

Table 5: Bioactive Properties of Multi-Whole Grain Mix

Bioactive properties	Multi-whole grain mix
In-vitro starch digestibility (%)	61.8 ± 1.7
Vitamin E activity (i.u./100g)	2.6 ± 0.2
Total antioxidant activity (mM alpha-Tocopherol Eq./g)	11 ± 0.1
Free radical scavenging activity (mg Catechin Eq./100g)	153 ± 8.0

Values are mean ± standard deviation of three independent determinations.

Polyphenols

The soluble, bound and total polyphenolic contents in multi-whole grain mix were 94, 132 and 226 mg GA Eq./100g, respectively. Main source of polyphenols in the mix is from finger mullet which is reported to contain 450mg/100g soluble and 900 mg/100g bound polyphenols [56], sorghum and maize [57]. Content in the mix is better than the refined grains were it is very low, 6 mg/100g, in the case of polished rice due to the loss of bran layers during polishing [58].

Bioactive Properties of Multi-Whole Grain Mix

Vitamin E Activity

The biological activity of vitamin E in animals is defined by its influence on symptoms of deficiency, including neuropathy, fetal death, or myopathy (muscle disease), and is dependent upon distinct regulatory processes. Vitamin E activity indicates the estimated biopotency of vitamin E and that in i.u. for alpha tocopherol 1.0; gamma tocopherol 0.25; and delta tocopherol 0.01 [59]. Vitamin E activity was 1.9 i.u./100 g in whole grain mix where as it is low in refined cereal and millet flours based on the vitamin E composition.

Total Antioxidant Capacity

The total antioxidant capacity of the mix was 17mM a-Tocopherol Eq./g. Vitamin E, carotenoids, and polyphenols in the food contribute in total antioxidant capacity. Investigations in our lab also have shown that finger millets are rich in polyphenols [55] and have free radical quenching ability [60]. Phenolics like ferulic acid and coumaric acid in cereals are known to express

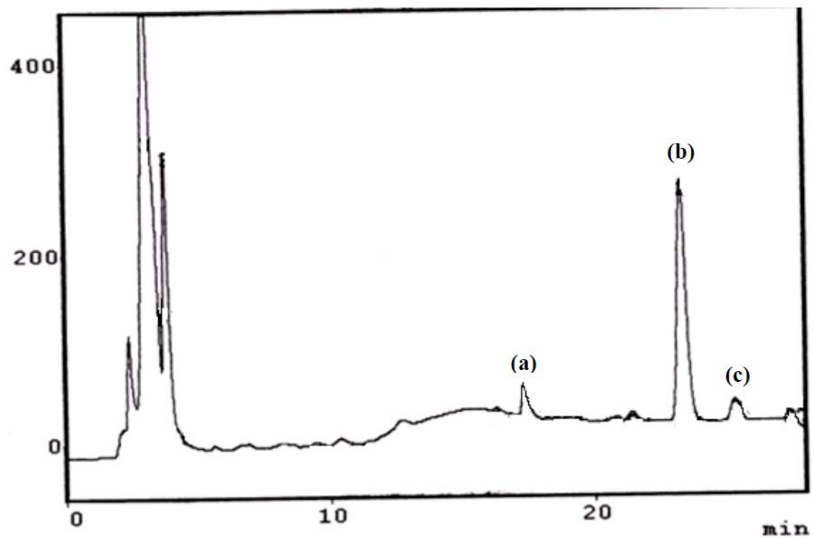

Figure 4: HPLC of Vitamin E in the mix showing gamma-tocotrienol (**a**), gamma-tocopherol (**b**) and alpha - tocopherol (**c**).

high antioxidant activity. These phytochemicals contribute to effective antioxidant potency. Other cereals like sorghum [61], wheat [62] and rice [63,64] are also reported to have antiradical properties. Polished rice contains only 3 mM a-T Eq. /g, which is much less than in the fully polished small millets [5]. As investigated by [65], the antioxidant properties like TPC, DPPH radical scavenging activity, oxygen radical absorbance capacity, ferulic acid content by HPLC analysis showed the evidence on the potential health benefits to be derived from consuming whole grain products.

DPPH Free Radical Scavenging Activity

The free-radical scavenging potentials of the methanolic extract of multi-whole grain mix were analyzed by the DPPH method, and the results are shown in (Figure **5**). It exhibited 33, 38, 42 and 45 % free-radical activity at the 20, 30, 40 and 50 μl of methanolic extract. Free radical scavenging activity of the mix was 153 mg CAT Eq. /100g and it is higher than refined grain products.The activity of the extract is attributed to its hydrogen-donating ability [66]. The antioxidants are believed to intercept the free radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups, there by forming a stable end product, which does not propagate further oxidation of the lipid [67].

In-Vitro Starch Digestibility

The starch digestibility of mix was 62 % and this medium digestibility is caused by partial gelatinization of the starches present in various grains which has undergone the process of roasting. Low digestibility in legumes is caused by the crystalline structure of starch, which protects the glucoside bonds and limits enzyme hydrolytic action [68]. Once fully gelatinized, this crystalline structure is lost, leaving the molecules open for hydrolysis, which breaks the glucoside bonds, and therefore increases digestibility. Low digestibility may also be due to the presence of fibre and polyphenols in whole grains which will reduce the digestibility of starch [69, 70]. Relatively lower carbohydrate digestibility makes the multi-whole grain mix suitable for diabetics also.

Sensory Quality of the Product

Sensory evaluation was carried out to make sure that the mix which is rich in nutrients and nutraceuticals is acceptable to the consumers. Scores for buff colour, consistency, flavors of toasted cereals and pulse, milk, cardamom were optimum in whole-multi grain drink and porridge samples (Figure **2**). Overall acceptability was 10 and 11, respectively for drink and porridge with sugar, and 7.5 and 7.7 without sugar. This indicates that the product can be used with and without sugar and can be consumed by young and adult as a source of macro and micronutrients and phytochemicals.

CONCLUSIONS

Ready to cook multi-whole grain mix is an ideal blend of cereals, millets, pulses and nuts which provide the benefits of nutrients and nutraceuticals present in different whole grains. Mix can be used for the preparation of drink or porridge with or without sugar and it was sensorily acceptable for all the age groups.

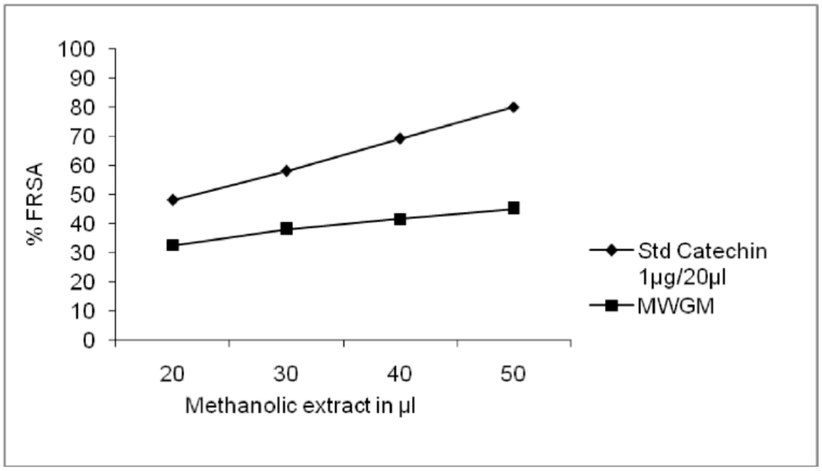

Figure 5: DPPH free radical scavenging activity (FRSA) of methanolic extract of multi-whole grain mix and the standard catechin.

Mix is rich in nutrients, nutraceuticals and bioactive properties compared to the refined grains. So this convenient, tasty multi-whole grain mix can be used as wholesome food for health and wellness.

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REFERENCES

[1] Slavin J. Whole grains and human health. Nutr Res Rev 2004; 17(1): 99-100. http://dx.doi.org/10.1079/NRR200374

[2] Gopalan C, Rama Sastri BV, Balasubramanian SC. Nutritive Value of Indian Foods. Hyderabad: National Institute of Nutrition, Indian Council of Medical Research 2004.

[3] Namiki M. Antioxidant/antimutagens in food. Crit Rev Food Sci Nutr 1990; 29: 273-300. http://dx.doi.org/10.1080/10408399009527528

[4] Gunger N, Sengual M. Antioxidant activity, total phenolics content and selected physicochemical properties of white mulberry (Morus *Alba* L.) fruits. Int J Food Properties 2008; 11: 44-52. http://dx.doi.org/10.1080/10942910701558652

[5] Asharani VT, Jayadeep A, Malleshi NG. Natural antioxidants in edible flours of selected small millets. Int J Food Properties 2010; 13: 41-50. http://dx.doi.org/10.1080/10942910802163105

[6] Stanner SA, Hughes J, Kelly CN, Buttriss J. A review of epidomiological evidence for the antioxidant hypothesis. Public Health Nutr 2004; 7: 407-22. http://dx.doi.org/10.1079/PHN2003543

[7] Flohé B, Traber MG. Vit amin E: function and metabolism. The FASEB J: Official Publication of the Federation of American Societies for Experimental Biology 1999; 13(10): 1145-55.

[8] Herrera, Barbas C. Vitamin E: action, metabolism and perspectives. J Physiol Biochem 2001; 57(2): 43-56.

[9] Muller DP. Vitamin E and neurological function. Review. Mol Nutr Food Res 2010; 54: 710-18. http://dx.doi.org/10.1002/mnfr.200900460

[10] Dowd P, Zheng ZB. On the mechanism of the anticlotting action of vitamin E quinone. Proc Natl Acad Sci USA 1995; 92: 8171-8175. http://dx.doi.org/10.1073/pnas.92.18.8171

[11] Devaraj S, Hugou I, Jialal I. Tocopherol decreases CD36 expression in human monocyte-derived macrophages. J Lipid Res 2001; 42: 521-27.

[12] Palozza P, Krinsky NI. Antioxidant effects of carotenoids and an overview. Methods Enzymol 1992; 213: 403-52. http://dx.doi.org/10.1016/0076-6879(92)13142-K

[13] Hinds TS, West WL, Knight EM. Carotenoids and retinoids - a review of research, clinical and public health applications. J Clin Pharmacol 1997; 37: 551-58.

[14] Beatty S, Boulton M, Koh HH, Murray IJ. Macular pigment and age related macular degradation. Br J Ophthalmol 1999; 83: 867-77. http://dx.doi.org/10.1136/bjo.83.7.867

[15] Christopher BJ. Carotenoids as colorants and Vitamin A precursors. In Carotenoids as food colors. Academic Press INC, New York 1981; pp. 150-152.

[16] Julia MH, Robin DG, Daryl JM. Application of reflectance colour measurement to the estimation of carotene and lutein content in wheat and triticale. J Cereal Sci 2004; 40: 151-59. http://dx.doi.org/10.1016/j.jcs.2004.07.005

[17] Krings V, El-Saharty Y, El-Zeany BA, Pabel B, Berger RG. Antioxidant activity of extracts from roasted wheat germ. Food Chemistry 2000; 71: 91-95. http://dx.doi.org/10.1016/S0308-8146(00)00148-5

[18] Mitsuru W. Antioxidative phenolic compounds from Japanese barnyard millet grains. J Agric Food Chem 1999; 47: 4500-505. http://dx.doi.org/10.1021/jf990498s

[19] Chethan S, Malleshi NG. Finger millet polyphenols: Characterization and their nutraceutical potential. Am J Food Technol 2007; 2: 582-92. http://dx.doi.org/10.3923/ajft.2007.582.592

[20] Guohua C, Eimin S, Ronald LP. Antioxidant and pro-oxidant behaviour of flavanoids. Structure activity relationships. Free Radic Biol Med 1997; 22: 749-60. http://dx.doi.org/10.1016/S0891-5849(96)00351-6

[21] Gavidel, Prakash. Composite weaning mixes: formulation and quality characteristics. Food Sci Technol Res 2010; 16(1): 65-70. http://dx.doi.org/10.3136/fstr.16.65

[22] Daodu, Chandrasekar. Development of weaning food formulations based on malting and roller drying of sorghum and cow pea. Int J Food Sci Technol 1989; 24, 511-19.

[23] Wang JC, Kinsella JE. Functional properties of novel proteins: Alfalfa leaf protein. J Food Sci 1976; 41: 286-92. http://dx.doi.org/10.1111/j.1365-2621.1976.tb00602.x

[24] Bhattacharya KR, Zakiuddin Ali S. A sedimentation test for pregelatinized rice products. Lebnsm.-Wiss. U.-Technology, 1976; 9: 36-37.

[25] Bong KK, Singh V. Cooking behavior of rice and black gram in the preparation of Idli, A traditional fermented product of Indian origin, by viscography. J Text Stud 2009; 40: 36-50. http://dx.doi.org/10.1111/j.1745-4603.2008.00168.x

[26] American Association of Cereal Chemists. Approved methods of AACC (10^{th} ed.). St Paul, Minnesota 2000.

[27] Association of Official Analytical Chemists. Official methods of AOAC (17th ed.). Washington, DC 2000.

[28] Asp NG, Johansson CG, Hallmer H, Siljestrom M. Rapidenzymatic assay of insoluble and soluble dietary fiber. J Agric Food Chem 1983; 31: 476-82. http://dx.doi.org/10.1021/jf00117a003

[29] Ranganna S. Plant pigment analysis and quality control for fruit and vegetable products. In Analysis of fruit and vegetable products, 2, Tata Mc graw Hill Publishing Company limited, New Delhi 1986; pp. 84-87.

[30] Kaplan LA, Miller JA, Stein EA, Stampfer MJ. Simultaneous HPLC analysis of retinol, tocopherols, lycopene and α-β-carotene in serum and plasma. Methods Enzymol 1990; 189: 155-67. http://dx.doi.org/10.1016/0076-6879(90)89286-Q

[31] Chen MH, Bergman CJ. A rapid procedure for analyzing rice bran tocopherol, tocotrienols and gama oryzanol contents. J Food Composit Anal 2005; 18, 312-31.

[32] Siwela, Muthulisi, Taylor, John RN, de Milliano, Walter AJ, Duodu, Kwaku G. Occurrence and Location of Tannins in Finger Millet Grain and Antioxidant Activity of Different Grain Types. Cereal Chem 2007; 84: 169-74.

[33] Singleton VL, Orthofer R, Rosa ML. Analysis of total phenols and other oxidation substrate and antioxidants by means of Folin–Ciocalteau reagent. Methods Enzymol 1995; 299: 152-71. http://dx.doi.org/10.1016/S0076-6879(99)99017-1

[34] Ngo Som J, Mouliswar P, Daniel, Malleshi NG, Venkat, Rao S. Digestibility of protein and starch in malted weaning foods. J Food Sci Technol 1992; 29: 262-63.

[35] Bernfeld P. Amylases α and β. Methods Enzymol 1990; 1: 149-50. http://dx.doi.org/10.1016/0076-6879(55)01021-5

[36] Pilar P, Manvel P, Mignel AI. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex. Anal Biochem 1999; 269: 337-41. http://dx.doi.org/10.1006/abio.1999.4019

[37] Bondet V, Williams B, Berset. Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. Lebnsm-Wiss U-Technol 1997; 30: 609-15.

[38] Stone H, Sidel TC. Quantitative Descriptive Analysis, developments, application and the future. Food Technol 1998; 52(8): 48-52.

[39] Snedecor GW, Cochran WG. Statistical methods. Iowa: Iowa State University Press 1994.

[40] Bello AB, Rooney LW, Waniska RD, Factors affecting quality of sorghum to, a thick porridge. Cereal Chem 1990; 67(1): 20-25.

[41] Hameeda B, Itagi N, Singh V. Preparation, nutritional composition, functional properties and antioxidant activities of multigrain composite mixes. J Food Sci Technol 2012; 49(1): 74-81. http://dx.doi.org/10.1007/s13197-011-0267-6

[42] Katti SV, Kumar S, Malleshi NG. Studies on the effect of milling finger millet in different pulverisers on physico-chemical properties of the flour. J Food Sci Technol 2008; 45(5): 398-405.

[43] Deepa G, Singh V, Naidu KA. Nutrient, Physico- chemical properties of Indian Medicinal Rice: Njavara. Food Chem 2008; 106(1): 165-71. http://dx.doi.org/10.1016/j.foodchem.2007.05.062

[44] Cheng HH. Total dietary fiber content of polished, brown and bran types of japonica and indica rice in taiwan - resulting physiological-effects of consumption. Nutr Res 1993; 13(1): 93-101. http://dx.doi.org/10.1016/S0271-5317(05)80660-8

[45] Barros F, Alviola JN, Rooney LW. Comparison of quality of refined and whole wheat tortillas. J Cereal Sci 2010; 51(1): 50-56. http://dx.doi.org/10.1016/j.jcs.2009.10.001

[46] Curic D, Galic K. Development of functional cereal based foodstuffs. Proceedings of the 3rd International Congress Flour - Bread '05 and 5th Croatian Congress of Cereal Technologists 2006; pp. 121-133.

[47] Indrani D, Swetha P, Soumya C, Rajiv J, Rao GV. Effect of multigrains on rheological, microstructural and quality characteristics of north Indian parotta - An Indian flat bread. Lwt-Food Sci Technol 2011; 44(3): 719-24. http://dx.doi.org/10.1016/j.lwt.2010.11.017

[48] Fardet A, Canlet C, Gottardi G, Lyan B, Llorach R, Remesy C, *et al*. Whole-grain and refined wheat flours show distinct metabolic profiles in rats as assessed by a H-1 NMR-based metabonomic approach. J Nutr 2007; 137(4): 923-29.

[49] Jones JM. Whole grains and dietary fiber continue to win honors in preventing various diseases. Cereal Foods World 2007; 52: 286-88.

[50] Scott CE, Eldrige AL. Comparison of carotenoid content in fresh, frozen and canned corn. J Food Composit Anal 2005; 18: 551-59. http://dx.doi.org/10.1016/j.jfca.2004.04.001

[51] Mamatha BS, Sangeetha RK, Baskaran V. Provitamin-A and xanthophyll carotenoids in vegetables and food grains of nutritional and medicinal importance. Int J Food Sci Technol 2011; 46: 315-25. http://dx.doi.org/10.1111/j.1365-2621.2010.02481.x

[52] Mares JA, Millen AE, Ficek TL, Hankinson SE. The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease: Overview. J Nutr 2002; 132: 518-24.

[53] Sakina K, Gopalakrishna AG. Fat soluble nutraceuticals and fatty acid composition of selected Indian rice varieties. J Am Oil Chem Soc 2004; 81: 939-43. http://dx.doi.org/10.1007/s11746-004-1005-5

[54] Wang, Quinn PJ. Vitamin E and its function in membranes. Progr lipid Res 1999; 38(4): 309-36. http://dx.doi.org/10.1016/S0163-7827(99)00008-9

[55] Xu Z, Hua N, Godber JS. Antioxidant activity of tocopherols, tocotrienols and γ-Oryzanol components from rice bran against cholesterol oxidation accelerated by 2,2'-Azobis (2-methlypropionamidine) Dihydrochloride. J Agric Food Chem 2001; 49: 2077-81. http://dx.doi.org/10.1021/jf0012852

[56] Chethan S, Malleshi NG. Finger millet polyphenols: Optimization of extraction and the effect of pH on their stability. Food Chem 2007; 105: 862-70. http://dx.doi.org/10.1016/j.foodchem.2007.02.012

[57] Awika JM, Lloyd W, Rooney. Sorghum phytochemicals and their potential impact on human health. Phytochemistry 2004; 65: 1199-21. http://dx.doi.org/10.1016/j.phytochem.2004.04.001

[58] Su T, Nakamura K, Kayahara H. Analysis of Phenolic Compounds in White Rice, Brown Rice, and Germinated Brown Rice. J Agric Food Chem 2004; 52: 4808-13. http://dx.doi.org/10.1021/jf049446f

[59] Grela ER, Gunter KD. Fatty acid composition and tocopherol content of some legume seeds. Animal Feed Technol 1995; 52: 325-31. http://dx.doi.org/10.1016/0377-8401(94)00733-P

[60] Sripriya G, Chandrasekharan K, Murthy VS, Chandra TS. ESR Spectroscopic studies on free radical quenching action of finger millet. Food Chem 1996; 57: 537-40. http://dx.doi.org/10.1016/S0308-8146(96)00187-2

[61] Vasudeva GK, Chandrashekar A, Rajani PS. Antiradical properties of sorghum flour extracts. J Cereal Sci 2004; 40: 283-88. http://dx.doi.org/10.1016/j.jcs.2004.08.004

[62] Ting S, Ho C. Antioxidant activities of buk wheat extracts. Food Chem 2005; 90: 743-49. http://dx.doi.org/10.1016/j.foodchem.2004.04.035

[63] Iqbal S, Bhanger MI, Farroq A. Antioxidant potential and components of some commercially available varieties of rice bran in Pakistan. Food Chem 2005; 93: 265-72. http://dx.doi.org/10.1016/j.foodchem.2004.09.024

[64] Jayadeep A, Malleshi NG. Nutritional and Nutraceutical Qualities of Biotransformed Rice, Cy TA. J Food 2010; 9: 82-87.

[65] Hirawan R, Ser WY, Arntfield SD, Beta T. Antioxidant properties of commercial, regular- and whole-wheat spaghetti. Food Chem 2010; 119(1): 258-64. http://dx.doi.org/10.1016/j.foodchem.2009.06.022

[66] Shimada KK, Fujikawa KY, Nakamura T. Antioxidative properties of xanthan on autoxidation of soybean oil in cyclodextrin. J Agric Food Chem 1992; 40: 945-48. http://dx.doi.org/10.1021/jf00018a005

[67] Sherwin ER. Oxidation and antioxidants in fat and oil processing. J Am Oil Chem Soc 1978; 55: 809-14. http://dx.doi.org/10.1007/BF02682653

[68] Ruiz-Ruiz J, Martinez-Ayala A, Dargo S, Gonzalez R, Betancur-Ancona D, Chel-Guerrero L. Extrusion of hard to cook bean (*Phaseolus vulgaris* L) and quality protein maize (*Zea mays* L.) flour blend. LWT – Food Sci Technol 2008; 41: 1799-807.

[69] Shobana S, Sreerama YN, Malleshi NG. Composition and enzyme inhibitory properties of finger millet *(Eleusine coracana L.)* seed coat phenolics: Mode of inhibition of [alpha]-glucosidase and pancreatic amylase. Food Chem 2009; 115: 1268-73. http://dx.doi.org/10.1016/j.foodchem.2009.01.042

[70] Yadav BS, Sharma A, Yadav RB. Effect of storage on resistant starch content and *in vitro* starch digestibility of some pressure-cooked cereals and legumes commonly used in India. Int J Food Sci Technol 2010; 45: 2449-55. http://dx.doi.org/10.1111/j.1365-2621.2010.02214.x

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