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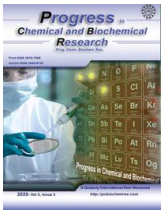

Original Research Article

Nutritional and Functional Properties of Amaranth Grain Flour Fractions Obtained by Differential Sieving

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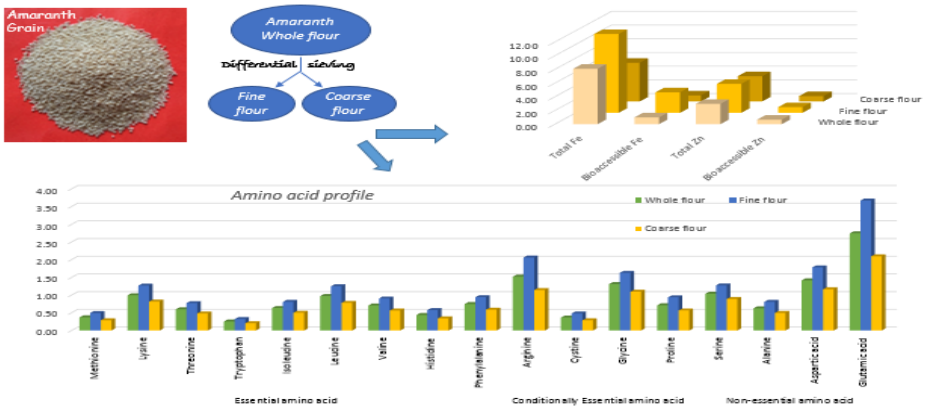
ABSTRACT

Amaranth grain, a gluten-free grain was milled to flour and differentially sieved to coarse and fine fractions. The whole flour and fractions thereof were analyzed for the nutrient composition, antinutrients, total and bioaccessible minerals, fatty acids, and amino acid profile and functional properties of flours. Results indicated that the fine fraction representing 44% of the whole amaranth flour contained higher protein (19.7%), fat (8.54%), minerals (3.46%) and dietary fibre content (20.09%) as well as a higher overall amino acid profile with lysine as its major essential amino acid. Linoleic acid (44.8%) in fine flour whereas oleic (29.4%) and palmitic acid (29.6%) in coarse flour was the predominant fatty acid found in amaranth flour fractions. Minerals were variedly distributed in analyzed fractions as iron was found majorly in fine flour and calcium in coarse flour. A similar trend in mineral bioaccessibility was observed. The *in vitro* protein digestibility of amaranth flour samples ranged from 59.8-72.5%. Functional properties revealed that higher values of water and oil absorption capacity were characterized in the coarse fraction, while whole flour showed higher foaming capacity and stability. Thus, differentially sieved flour fractions of amaranth grain showed a wide distribution of nutrients and in particular, the finer fraction was nutrient-dense. It was found to be an excellent source of nutrients and could be incorporated as a functional ingredient in the development of nutrient-rich products.

GRAPHICAL ABSTRACT



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1. Introduction

Amaranth belongs to family *Amaranthaceae* and the word 'Amaranthus' basically is derived from the Greek word "Anthos" (flower) which means everlasting or unwilting. The grain amaranth is one among the major pseudocereal grains, which do not belong to the grass family but produce fruits and seeds. This crop has a great amount of genetic diversity and has the ability to produce more than one phenotype upon different environment exposure. With an extreme capacity to adapt to adverse growing conditions, the crop can resist heat and drought, has no major disease reported, and can be easily grown in any agricultural land [1].

Nutritionally, amaranth has higher protein content than that of most cereals and has been reported as an important source of bioactive components [2]. The bioactive peptides derived from amaranth protein have shown many bio-functionalities such as antimicrobial, antioxidant, antihypertensive activities [3, 4], and anticancer activity [5]. Amaranth protein is also associated with potential hypocholesterolemic effect and its pure peptides have an anti-atherosclerotic effect [6]. Amaranth also exerts soluble peptides which showed anti-inflammatory effects in colonic epithelial cells and therefore raised the use of amaranth peptides as a potential dietary supplement [7]. Thus, this ancient grain is gaining much importance in the present scenario for its rich nutritional content and various health-promoting properties. Additionally, amaranth flour presents good film-forming ability and yields films with moderate solubility, high flexibility, and excellent water vapour barrier properties. These edible films consist of biopolymers that can be used in food packaging [8].

Milling represents the principle procedure in the food industry, wherein the outcome-products are further used for the development of finished products. In milling processes, one of the categories is dry milling which separates the outer fibrous material (seed coat, aleurone, and sub-aleurone layers) and germ by abrasive technique to obtain polished grain and by-product with a high concentration of bioactive compounds [9]. Other investigations also report the influence of particle size (74 - 210µm) on the compositional, functional, and rheological properties of lentil flour. And the results obtained could be beneficial for food industries to manufacture quality food products with desired functional and rheological characteristics using defined particle size or fractions [10]. Milled flour is a combination of fine and coarse flour with varying nutrient composition and availability which are bound to the grain matrix. Our earlier studies on differential milling of various cereals such as wheat, pearl millet, finger millet, and sorghum have shown differences in nutritional composition and antioxidant activity [11-13]. Hence, the present study was undertaken with the objective of separating fractions from whole amaranth flour by differential sieving and analyzing these fractions along with whole flour for nutritional and functional properties. Further, the *in vitro* nutrient availability for

selected nutrients was measured in all flour samples.

2. Experimental

2.1. Materials

Amaranth grain (*Amaranthus caudatus*) was procured in a single lot from a local certified organic shop. The analytical grade (AR grade) chemicals, solvents, and acids used throughout the experiments were procured from Sisco Research Laboratories Pvt Ltd (SRL), Sd Fine-chem Ltd and Merck Life Science Pvt Ltd, Mumbai, India. In the study, enzymes used were pepsin (GRM9155), pancreatin (RM3867), and bile salt from Himedia, α- amylase from *Bacillus amyloliquefaciens* (Lot#SLBF7325V) from Sigma-Aldrich chemicals Pvt Ltd. The dialysis tube (LA395) with specification 31.71 mm width, 21.5 mm diameter, 2.4 nm pore size and molecular cut off between 12,000 to 14,000 were procured from Himedia Co.

2.2. Methods

The amaranth grain was cleaned, dried in the hot-air oven at 40°C for 4 hr, and milled into flour in plate mill. The process of differential sieving was carried out by passing the milled whole flour through a standard 100 mesh sieve (149 μm) to separate into two fractions i.e., fine flour and coarse flour. The yield of each flour fraction after differential milling was recorded as 44% fine flour and 56% coarse flour. All the flours were stored in air-tight zip-lock pouches at 4°C until further analysis. A portion of whole flour was retained as such, which served as control.

2.2.1. Analysis

For analysis, differentially sieved flour i.e. fine flour (FF) and coarse flour (CF) along with whole amaranth flour (WF) were analyzed for nutritional composition, antinutrient and mineral content, amino acid, and fatty acid profile and functional properties. The *in vitro* studies such as protein digestibility and mineral bioaccessibility was also investigated in these flours. The same batch of flour was used in each experiment and all samples were analyzed in triplicates in the following analysis. Triple distilled water was used in mineral estimation and *in vitro* studies.

Nutrient composition

Differentially sieved flour fraction and WF were determined for moisture content by oven drying method, total fat content by solvent extraction by Soxhlet method, and protein by Kjeldahl method and the nitrogen value was multiplied with factor 6.25 to obtain protein content [14]. The soluble, insoluble, and total dietary fibre contents were estimated by rapid enzymatic assay [15]. The carbohydrate content was calculated by difference.

Antinutritional content

The phytic acid was estimated by the method of Thompson and Erdman [16]. Phosphorous was converted into phytic acid using 3.55 as a conversion factor. Condensed tannins extracted from flour fractions using methanol were analyzed colorimetrically by the vanillin-HCl method and results expressed as milligrams of (+)-catechin equivalents per 100g [17].

In vitro protein digestibility

Akeson and Stahman [18] method were followed to determine *in vitro* digestible protein in amaranth flour fractions. Sample containing 100 mg protein was digested with enzyme pepsin and pancreatin in required pH adjustment. The insoluble protein was separated using trichloroacetic acid and the soluble protein was centrifuged and the supernatant was digested in acid to estimate the protein by Kjeldahl method. Protein digestibility results are expressed as a percent of total protein solubilized after enzyme hydrolysis.

Mineral content and its Bioaccessibility

The mineral content of flour samples was analyzed by ashing the flour in a muffle, later the ash was digested in concentrated HCl over the water bath. The process was repeated twice and further with few drops of triple distilled water added, the solution was filtered through ashless filter paper and made-up to a known volume. The minerals quantified in ash solution were iron, zinc, calcium, magnesium, manganese, potassium, sodium, phosphorous, and copper [14]. The bioaccessibility of mineral in these flours was also analyzed by the *in vitro* method of dialysis by simulating gastrointestinal digestion [19]. The dialysates were estimated for dialyzable minerals, specifically iron, calcium, and zinc. The mineral concentration in the prepared ash solution and dialysates was measured in Inductively coupled plasma optical emission spectrometer (ICP-OES) (Perkin Elmer Optima™ 8300, MA, USA). This spectrometer instrument is an elemental analysis technique that identifies and quantifies elements present in trace-level using emission spectra of a sample. Percent bioaccessibility of the minerals was computed by comparing the dialyzable mineral with the total mineral present in the flour.

Amino acid Profile

The amino acid present in amaranth flour fraction was determined by ion chromatography in an Amino acid analyzer released by hydrolysis with boiling semi-concentrated hydrochloric acid. Biochrom 30+ series of Amion acid analyzer from Biochrom Ltd. Cambridge, UK with two-channel A/D converter card on a PC using Software for peak integration was the instrument used. The aminograms were detected at 570 nm and 440 nm [20].

Fatty acid profile

Liang [21] method was carried out to analyze the fatty acid profile of amaranth flour fractions. The fatty acid was measured in gas chromatography (GC) fitted with a capillary column and flame ionization detector into which fatty acid methyl esters were fed. GC model used - GC-2010plus, Shimadzu Corporation, Tokyo, Japan, and column used was 30m x 0.25mm ID, DF-0.25µm thickness. The injector and detector temperature were maintained at 260 - 280°C. The fatty acids were identified and quantified with reference to the retention time of standard fatty acid methyl esters under similar conditions and expressed as a percentage of relative area.

Functional properties

To determine the functional properties, the analyzed flours were dried at 40°C for 24hrs

to reach equilibrium moisture. Water absorption capacity (WAC) and water solubility index (WSI) of the differentially sieved fractions and whole flour was determined by following the procedure reported by Elhardallou & Walker [22] and Anderson, Conway and Griffin, [23] respectively. WAC is reported as g of water absorbed/100g of dry flour and the weight ratio of dissolved solids in the supernatant and dry sample reports WSI. Oil absorption capacity (OAC) is the measure of oil taken up by the known amount of flour sample and reported as g of oil absorbed/100g of dry flour [24]. Coffmann and Garcia, [25] method was followed to estimate foaming capacity and stability in the flour fractions.

2.3. Statistical analysis

Each experiment data was presented as means ± standard deviation of three concordant measurements. The significant difference comparisons were done by one-way analysis of variance (ANOVA), followed by the T-test in SPSS 16.0 [26] and the statistical significance was defined as $P < 0.05$.

3. Results and Discussion

3.1. Nutritional composition

The nutritional composition, antinutritional content, and *invitro* digestible protein of experimental flours are presented in table 1. The analyzed flour with approximately 7 - 9% moisture content showed nearly 15-20% of protein and dietary fibre as major nutrients apart from carbohydrates. Each flour fractions differed significantly in terms of protein, fat, mineral, carbohydrate, and total dietary fibre content. FF showed higher content of protein (19.7%), fat (8.5%), and minerals (3.5%) and lower carbohydrate content than WF followed by CF on a dry weight basis (*dw*). Similar values of nutrient content in amaranth WF were reported by Menegassi, Pilosof, and Areas, [27] and Sanz-Penella, Wornkowska, Smietana, and Haros, [28]. Another study by Kumar, Dharmaraj, Sakhare, and Inamdar, [29] on fractionation to develop protein and mineral-rich fraction from amaranth grain, observed higher nutrient content in seed coat coarser fraction than the finer fraction and the whole flour. Amaranth grain contains high-quality protein essentially composed of globulin and albumin with less glutamic acid and proline than prolamins, the absence of gluten makes it a major gluten-free grain for incorporation into the diet of celiac disease patients [30]. And the composite flour developed by Twinomuhwezi, Awuchi, and Rachael, [31] by combining amaranth, rice, soybean, and millets states the use of rice-amaranth mixture as complimentary food due to its low bulk density and good proximate composition. The total dietary fibre was highest in FF (20.1%) followed by WF (15.3%) and CF (12.3%). CF had a higher proportion of IDF, whereas FF and WF were rich in SDF. A recent study also reports that amaranth provides a higher proportion of soluble fibre than true cereals like wheat and maize which is composed of branched xyloglucans with the majority of di- and trisaccharide side chains, as well as pectin polysaccharides [32].

The phytic acid content in flour fractions ranged from 1.43 – 2.42 g/100g *dw* with no

significant difference in WF and CF. The value of phytic acid in WF is similar to the content reported by Akin-Idowu, Ademoyegun, Olagunju, Aduloju, and Adebo, [33]. Condensed tannins are present in good quantity in the amaranth grain flour, wherein CF had the highest and FF had the lowest contents. The IVDP assay is a widely used method to determine the digestibility parameter by mimicking the simulated digestive processes in the human gastrointestinal tract through the pepsin-pancreatin enzyme system to measure the percentage of proteins hydrolyzed by digestive enzymes [34]. Amongst the fraction, the protein digestibility was highest in FF accounting for 72.5% of total protein, followed by CF (66.7%) and WF (59.8%).

Table 1- Chemical composition of Amaranth flour fractions per 100g

Constituent	***Flour fractions***		
	Whole	***Fine***	***Coarse***
Moisture (g)	7.94±0.07^{c}	8.43±0.07^{b}	8.92±0.06^{a}
Protein (g)	14.7±0.17^{b} (17.06)	16.5±0.18^{a} (19.7)	10.4±0.17^{c} (12.08)
Fat (g)	5.83±0.03^{b} (6.33)	7.82±0.06^{a} (8.54)	5.18±0.04^{c} (5.69)
Minerals (g)	2.26±0.02^{b} (2.62)	2.90±0.05^{a}	1.87±0.01^{c} (2.18)
CHO (by difference) (g)	50.8	39.8	58.8
Dietary fiber			
Total (g)	13.2±0.13^{b} (15.29)	16.8±0.96^{a} (20.09)	10.6±0.36^{c} (12.3)
Insoluble (g)	8.6±0.13^{bc} (10.03)	10.8±0.36^{a} (12.85)	8.6±0.23^{b} (10.05)
Soluble (g)	4.5±0.13^{b} (5.26)	6.1±0.6^{a} (7.24)	1.9±0.27^{c} (2.25)
Antinutrients			
Phytic acid (g)	1.37±0.06^{b} (1.59)	2.03±0.11^{a} (2.42)	1.23±0.05^{bc} (1.43)
Condensed Tannins (mg)	61.4±0.8^{b} (71.2)	42.1±1.7^{c} (50.3)	64.8±1.3^{a} (75.4)
In vitro digestible Protein			
Content (g)	10.2±0.22^{b} (11.8)	14.3±0.22^{a} (17.1)	8.1±0.38^{c} (9.4)
Percent (%)	59.8	72.5	66.7

Values reported are mean±standard deviation of triplicate determinations. Mean values with different alphabetical superscripts are significantly different within a row at $p<0.05$. Figures in the parenthesis represent values on dry weight (*dw*) basis.

3.2. Mineral content and mineral bioaccessibility

The mineral content and its bioaccessibility of flour samples are compiled in table 2. The concentration of Fe and its bioaccessibility was higher in FF i.e. 11.5 mg and 2.99 mg/100g *dw* respectively, and nearly half of it was observed in CF (5.65 mg/100g *dw*) with bioaccessibility of 0.89 mg/100g *dw*. The Zn content and its bioaccessibility in flour fractions ranged from 2.97 – 4.23 mg and 0.66 – 0.88 mg/100g *dw* respectively, with no significant difference between Zn contents of WF & CF. However, in the case of Ca, CF exhibited higher content (210 mg/100g *dw*) with 40% bioaccessibility (figure 1). It may be noted that among all minerals analyzed, CF was richer only in calcium content, the difference being significant between flour samples. Alvarez-

Jubete, Arendt, and Gallagher, [35] in their review on pseudocereals nutritive value and their increasing use as functional gluten-free ingredients observe that amaranth, quinoa, and buckwheat are good sources of important minerals and in particular they highlight amaranth grain for extremely higher mineral content (Fe, Ca, Zn and Mg) than other pseudocereals and true cereal. Similarly, comparing amaranth with the pulse, millet, and oilseeds reports higher mineral content particularly Fe and Zn [36] The mineral content reported in the present study is in agreement with values reported in their review and are also comparable with other studies [28, 37]. The calcium present in amaranth grain can serve as a crucial source of supplement and exerts anti-diabetic properties by improving calcium homeostasis in blood, kidney, and liver as described by Kasozi, Namubiru, Safiriyu, Ninsiima, Nakimbugwe, Namayanja, and Valladares, [38] in their study on the association of amaranth grain with improved hepatic and renal calcium metabolism in Type 2 diabetes mellitus of male Wistar rats. Further analyzed minerals i.e. Mn, Mg, K, P, and Cu showed a significant difference among the flour fractions presenting higher content in FF. Amaranth flour fractions showed notable amount of P (381 – 729 mg) > K (358 –620 mg) > Mg (219 – 393 mg) per 100g *dw*. Therefore, partial replacement of wheat flour with whole amaranth flour in bread formulation increased 2-3 fold of both micro- and macro-elements as reported by Sanz-Penella, Wronkowska, Soral-Smietana, and Haros [28].

Table 2- Mineral content of Amaranth flour fractions

Minerals	***Flour fractions***		
	Whole	***Fine***	***Coarse***
Total mineral content (mg/100g)			
Fe	6.99±0.14^{b} (8.1)	9.64±0.14^{a} (11.51)	4.85±0.22^{c} (5.65)
Zn	2.56±0.52^{bc} (2.97)	3.55±0.13^{a} (4.23)	3.17±0.24^{b} (3.69)
Ca	157±5.6^{bc} (182)	159±4.6^{b} (190)	180±6.4^{a} (210)
Mn	2.20±0.02^{b} (2.56)	2.57±0.01^{a} (3.07)	1.91±0.08^{c} (2.23)
Mg	249±3.7^{b} (289)	329±7.2^{a} (393)	189±8.0^{c} (219)
K	411±2.8^{b} (477)	520±11.2^{a} (620)	307±5.4^{c} (358)
P	443±5.1^{b} (513)	610±10^{a} (729)	327±12.8^{c} (381)
Cu	0.51±0.02^{c} (0.59)	0.73±0.06^{a} (0.87)	0.54±0.09^{b} (0.62)
Na	5.20±0.76^{a} (6.03)	4.69±0.44^{ab} (5.6)	4.68±0.42^{abc} (5.44)
Bioaccessible minerals (mg/100g)			
Fe	0.86±0.01^{b} (1.00)	2.51±0.01^{a} (2.99)	0.76±0.01^{c} (0.89)
Zn	0.57±0.0^{c} (0.66)	0.69±0.0^{a} (0.83)	0.67±0.0^{b} (0.78)
Ca	51±0.12^{b} (59)	43±0.01^{c} (52)	73±0.01^{a} (85)

Values reported are mean ± standard deviation of triplicate determinations. Mean values with different alphabetical superscripts were significantly different within a row at $p<0.05$ and figures in the parenthesis represent values in dry weight (*dw*) basis

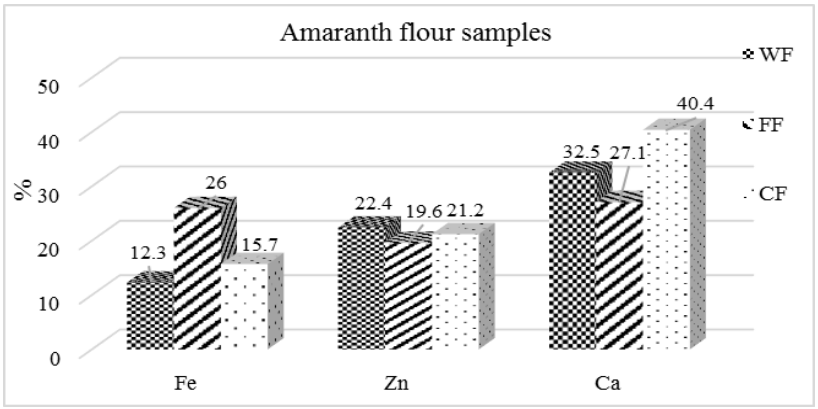

Fig. 1 Percent mineral bioaccessibility in amaranth flour samples

3.3. Fatty acid profile

Table 3 presents the fatty acid profile of amaranth flour samples. The predominant fatty acid found in amaranth grain was linoleic acid ranging from 37.2 – 44.8% which is a polyunsaturated fatty acid (PUFA), oleic acid 27.9 – 29.4% being a monounsaturated fatty acid (MUFA) and palmitic acid 26.5 – 29.6%, a saturated fatty acid. Another saturated fatty acid present in smaller amounts was stearic acid ranging from 5.97 – 7.09% *dw*. On comparing between amaranth flour fractions, CF constituted higher amount of palmitic acid (29.6%), stearic acid (7.09%), oleic acid (29.4%), linolenic acid (1.01%), arachidic acid (1.91%), and behenic acid (2.2%) whereas FF showed more of linoleic acid (44.8%) on *dw*. The stearic acid, linolenic acid, arachidic acid, and palmitoleic acid content present in WF and FF were not significantly different. These values are nearly similar with those found by Tang, Li, Chen, Zhang, Liu, Hernandez, Draves, Marcone, and Tsao, [39], reporting C18:2*ω-6* as the main PUFA (37.11-45.92%) and MUFA C18:1 was the second prominent fatty acid ranging from 22.72 -31.76% in different varieties of amaranth analyzed. In our study, fatty acids which are present in lower amount are linolenic acid (0.58 – 1.01% *dw*), palmitoleic acid (0.5 – 0.6% *dw*) and myristic acid (0.41-0.77% *dw*). Our results are also similar to reported values by Shukla, Srivastava, Suneja, Yadav, Hussain, Rana, and Yadav, [40]. Amaranth flour fraction also displayed a small amount of omega-3 fatty acid i.e. Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). The EPA content was highest in FF (3.43% *dw*) and lowest in CF (1.45% *dw*) and vice versa in the case of DHA i.e. CF has the highest content (1.45% *dw*) and least in FF (1.13% *dw*). So far, no literature study has reported EPA and DHA content in the fatty acid profile of amaranth flour. Thus, it can be said that amaranth provides essential fatty acids in a significant amount and imparts various health beneficiary effects.

WHO, [41] reports the nutritional importance of essential amino acids (EAA) as they cannot be synthesized in the human body, and must be provided through diet. They are most important for the growth and maintenance of metabolic needs than non-essential amino acids. Apparently, another

category called 'conditionally essential' amino acids in which the human body cannot synthesize in the adequate amount under specific physiological or pathological conditions needs to be taken through diet only. The quantified amino acid profile of amaranth flour samples is represented in table 4, which shows a varying quantity of EAA in different samples. Lysine and leucine are the amino acids present in higher amounts among the EAA ranging from 0.818 – 1.269% and 0.781-1.25% *dw* respectively.

Table 3: Fatty acid profile of Amaranth flour fractions (%)

	Flour fractions		
	Whole	***Fine***	***Coarse***
Myristic acid (C14:0)	0.66 ± 0.01^{a} (0.77)	0.35 ± 0.01^{c} (0.41)	0.45 ± 0.03^{b} (0.52)
Palmitic Acid (C16:0)	22.9 ± 0.02^{c} (26.5)	23.1 ± 0.08^{b} (27.6)	25.5 ± 0.06^{a} (29.6)
Palmitoleic Acid (C16:1)	0.43 ± 0.01^{bc} (0.5)	0.50 ± 0.02^{a} (0.6)	0.51 ± 0.01^{b} (0.59)
Stearic Acid (C18:0)	5.15 ± 0.03^{bc} (5.97)	5.02 ± 0.05^{b} (6.0)	6.09 ± 0.02^{a} (7.09)
Oleic Acid (C18:1)	24.0 ± 0.09^{c} (27.9)	24.2 ± 0.05^{b} (28.9)	25.3 ± 0.03^{a} (29.4)
Linoleic Acid (C18:2)	37.7 ± 0.03^{b} (43.7)	37.5 ± 0.05^{a} (44.8)	31.9 ± 0.05^{c} (37.2)
Linolenic Acid (C18:3)	0.50 ± 0.03^{bc} (0.58)	0.53 ± 0.04^{b} (0.63)	0.87 ± 0.04^{a} (1.01)
Arachidic Acid (C20:0)	1.25 ± 0.02^{bc} (1.45)	1.23 ± 0.07^{b} (1.47)	1.64 ± 0.01^{a} (1.91)
Behenic Acid (C22:0)	1.62 ± 0.02^{b} (1.87)	1.56 ± 0.05^{bc} (1.86)	1.89 ± 0.01^{a} (2.2)
EPA (C20:5)	2.03 ± 0.02^{b} (2.35)	2.87 ± 0.02^{a} (3.43)	1.81 ± 0.01^{c} (2.11)
DHA (C22:6)	1.05 ± 0.03^{b} (1.22)	0.94 ± 0.02^{c} (1.13)	1.24 ± 0.01^{a} (1.45)
Others	2.64 ± 0.01^{b} (3.06)	2.09 ± 0.01^{c} (2.5)	2.77 ± 0.01^{a} (3.23)

Values reported are mean±standard deviation of triplicate determinations. Mean values with different alphabetical superscripts were significantly different within a row at $p<0.05$ and figures in the parenthesis represent values in dry weight (*dw*) basis

3.4. Amino acid profile

Next in the series are phenylalanine, valine, and isoleucine present in higher quantity in FF of 0.924, 0.904, and 0.808% respectively and nearly half of this content is present in CF. Lastly, methionine, tryptophan, and histidine are present in lower content between the flour fractions. Overall, FF has a higher content of EAA of 7.34% followed by 5.73% in WF and 4.57% in CF on *dw* basis. Other studies have also observed the presence of higher EAA in amaranth flour in comparison with cornflour [42] and other conventional cereals [40]. Due to this reason, the incorporation of amaranth flour in food products enhances the content of EAA. In particular, it was seen that amaranth

incorporation increased 50% of lysine content in *chapatti (Indian flatbread)* fortified with 40% amaranth whole flour as compared with the control whole wheat *chapatti* [43]. It is also reported that lysine content in amaranth meets the daily requirement of infants, children, and adults. Arginine and glycine are among the conditionally EAA present in a higher amount than cystine and proline. In non-essential amino acid, glutamic acid is present in a higher amount in amaranth flour fractions ranging from 3.671 – 2.097% and it also forms a greater proportion of total amino acid content. Overall, the amaranth FF fraction separated by differential sieving represented a rich source of individual amino acids, both essential and non-essential, and had a much higher content than present in WF and CF.

3.5. Functional properties

Values in table 5 represent the functional properties of amaranth flour samples.

The CF obtained from differential sieving showed higher WAC of 174.8±1.2 g and FF showed the lowest of 122.2±0.7 g/100g *dw* conversely the WSI was higher in FF (12.2±0.3 g) and least in CF (5±0.3 g).

Table 4: Amino acid profile of Amaranth flour fractions (%)

	Flour fractions		
	Whole	***Fine***	***Coarse***
Essential amino acid			
Methionine	0.322±0^{b} (0.373)	0.413±0^{a} (0.493)	0.249±0^{c} (0.290)
Lysine	0.858±0^{b} (0.995)	1.063±0^{a} (1.269)	0.703±0^{c} (0.818)
Threonine	0.517±0^{b} (0.6)	0.648±0^{a} (0.774)	0.414±0^{c} (0.482)
Tryptophan	0.219±0^{b} (0.254)	0.271±0^{a} (0.324)	0.176±0^{c} (0.205)
Isoleucine	0.548±0^{b} (0.635)	0.677±0^{a} (0.808)	0.431±0^{c} (0.502)
Leucine	0.840±0^{b} (0.974)	1.047±0^{a} (1.25)	0.671±0^{c} (0.781)
Valine	0.611±0^{b} (0.709)	0.757±0^{a} (0.904)	0.484±0^{c} (0.563)
Histidine	0.379±0^{b} (0.44)	0.482±0^{a} (0.576)	0.295±0^{c} (0.343)
Phenylalanine	0.646±0^{b} (0.749)	0.789±0^{a} (0.942)	0.506±0^{c} (0.589)
Conditionally Essential amino acid			
Arginine	1.312±0^{b} (1.521)	1.725±0^{a} (2.06)	0.981±0^{c} (1.142)
Cystine	0.317±0^{b} (0.368)	0.402±0^{a} (0.48)	0.248±0^{c} (0.289)
Glycine	1.130±0^{b} (1.311)	1.363±0^{a} (1.627)	0.942±0^{c} (1.096)
Proline	0.615±0^{b} (0.713)	0.785±0^{a} (0.938)	0.487±0^{c} (0.567)
Non-essential amino acid			
Serine	0.897±0^{b} (1.04)	1.067±0^{a} (1.274)	0.765±0^{c} (0.89)
Alanine	0.540±0^{b} (0.626)	0.677±0^{a} (0.808)	0.425±0^{c} (0.495)
Aspartic acid	1.223±0^{b} (1.418)	1.494±0^{a} (1.783)	1.002±0^{c} (1.166)
Glutamic acid	2.367±0^{b} (2.745)	3.074±0^{a} (3.671)	1.801±0^{c} (2.097)
Methionine+cystine	0.639±0^{b} (0.741)	0.815±0^{a} (0.973)	0.497±0^{c} (0.579)
Total Amino acid	13.98 (16.2)	17.55 (20.95)	11.08 (12.89)

Values reported are mean±standard deviation of triplicate determinations. Mean values with different alphabetical superscripts were significantly different within a row at $p<0.05$. Figures in parenthesis represent values on dry weight (*dw*) basis.

Banerji et al., [43] found that WAC decreases with the increase in the addition of amaranth flour at 20 -50% level from 1.19 to 1.07 g/g, however, other researchers

observed the opposite. This difference could be for the fact that particle size, extraction rate, or composition varies in different flours based on its nutrient distribution or composition. The oil absorption capacity was lowest in FF and did not show any significant differences between WF ranging from 85.6 – 87.1 g/100g *dw* and reports highest in CF (89.1±05 g). The study by Naik, Dachana, Ramesh and Prakash, [44] reports a similar trend on analyzing fractionally milled finger millet flour in which overall functional capacities of coarser fraction flour were higher than the finer flour fraction. Likewise study on evaluating the roller milling potential of amaranth grains by Sakhare, Inamdar, Kumar, and Dharmaraj, [45] showed roller milled amaranth flour produced fractions rich in nutrients with unique functional properties. The highest foaming capacity was observed in WF of 8±0.73 ml/100g and nearly half of it was reported in both FF and WF which did not show any significant differences. A similar trend was observed upon setting aside the foam formed by these analyzed flours for 30-60 mins showed a slight drop in the stability. Bolontrade, Scilingo, and Aňón, [46] reports foams made with amaranth proteins at acidic pH and low ionic strength exhibit stability and form more flexible and elastic film. Therefore, these properties provide the opportunity to incorporate amaranth proteins into the development of foam-type foods such as dessert stuffing and ice creams.

Table 5 Functional properties of Amaranth flour fractions (%)

Functional property		***Flour fractions***		
		Whole	***Fine***	***Coarse***
Water absorption capacity (g/100g)		151.3±0.7^{b}	122.2±0.7^{c}	174.8±1.2^{a}
Water solubility index (g/100g)		8.2±0.4^{b}	12.2±0.3^{a}	5±0.3^{c}
Oil absorption capacity (g/100g)		87.1±1.1^{b}	85.6±1.4^{bc}	89.1±0.5^{a}
Foaming capacity (ml/100ml)		8±0.73^{a}	4.65±0.84^{b}	4.31±0.24^{bc}
Foaming stability (ml/100ml)	30 min	7.24±0.58^{a}	4.63±0.94^{b}	4.01±0.12^{bc}
	60 min	7.24±0.58^{a}	4.31±0.24^{b}	3.95±0.24^{bc}

Values reported are mean±standard deviation of triplicate determinations. Mean values with different alphabetical superscripts were significantly different within a row at $p<0.05$.

4. Concluding remarks

The present investigation demonstrates that FF contained higher protein, dietary fibre content, and mineral particularly, Fe and Zn with greater bioaccessibility than WF and CF. The Ca content and bioaccessibility of CF was reported higher. The fat content of amaranth flour samples

was 2-3 fold higher than true cereals. The fatty acids of amaranth flours constituted a high proportion of essential fatty acids, majorly unsaturated i.e. linoleic acid present in higher proportion in FF. Contrarily, CF had higher saturated fatty acids and MUFA than other samples. Analyzed flours also exhibited smaller *omega-3* fatty acids (1-3%). The higher protein quality and digestibility in FF showed balanced amino acid composition, especially greater in lysine and glutamic acid content among essential and non-essential amino acids respectively. Thus, the study indicates the potential utilization of amaranth flour fractions as functional ingredients for the development of nutrient-dense dietary supplements suitable for different age groups. The flours could also be used for their unique functional properties.

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Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCE

[1] A. Sharma, Amaranth: A Pseudocereal. *Nutrition & Food Science*, 3 (2017) 1-3.

[2] A. Rastogi and S. Shukla, Amaranth: A new millennium crop of nutraceutical values. *Critical Review in Food Science and Nutrition*, 53 (2013) 109-125.

[3] V.M. Caselato-Sousa and J. Amaya-Farfán, State of knowledge on amaranth grain: a comprehensive review. *Journal of Food Science*, 77 (2012) 93-104.

[4] A.V. Quiroga, P. Aphalo, J.L. Ventureira, E.N. Martínez and M.C. Añón, Physicochemical, functional and angiotensin-converting enzyme inhibitory properties of Amaranth (*Amaranthus hypochondriacus*) 7S globulin. *Journal of the Science of Food and Agriculture*, 92 (2012) 397-403.

[5] M.S. Taniya, M.V. Reshma, P.S. Shanimol, G. Krishnan and S. Priya, Bioactive peptides from amaranth seed protein hydrolysates induced apoptosis and antimigratory effects in breast cancer cells. *Food Bioscience*, 35 (2020) 100588.

[6] A. Montoya-Rodriguez and E.G. Mejía, Pure peptides from amaranth (*Amaranthus hypochondriacus*) proteins inhibit LOX-1 receptor and cellular markers associated with atherosclerosis development *in vitro*. *Food Research International*, 77 (2015) 204-214.

[7] J. Moronta, P.L. Smaldini, G.H. Docena and M.C. Añón, Peptides of amaranth were targeted as containing sequences with potential anti-inflammatory properties. *Journal of Functional Foods*, 21 (2016) 463-473.

[8] M.C.V. Diéguez, F.M. Pelissari, P.J.A. Sobral and F.C. Menegalli, Effect of process conditions on the production of nanocomposite films based on amaranth flour and montmorillonite. *LWT-Food Science and Technology*, 61 (2015) 70-79.

[9] M. Papageorgiou and A. Skendi, 1 – Introduction to cereal processing and by-products. Sustainable recovery and reutilization of cereal processing by-products. Woodhead Publishing Elsevier Ltd, United Kingdom, (2018) 1-26.

[10] J. Ahmed, A. Taher, M.Z. Mulla, Al-A. Hazza and G. Luciano, Effect of sieve particle size on functional, thermal, rheological and pasting properties of Indian and Turkish lentil flour. *Journal of Food Engineering*, 186 (2016) 34-41.

[11] M. Oghbaei and J. Prakash, Effect of fractional milling of wheat on nutritional quality of milled fractions. *Trends in Carbohydrate Research,* 5 (2013) 53-58.

[12] M. Oghbaei and J. Prakash, Effect of primary processing of cereals and legumes on its nutritional quality: A comprehensive review. *Cogent: Food and Agriculture*, 2 (2016) 1-14.

[13] A. Siddiq and J. Prakash, Antioxidant properties of digestive enzyme treated fiber rich fractions from wheat, finger millet, pearl millet and sorghum: A comparative evaluation. Cogent: Food and Agriculture, 1: 1073815 (2015) 1-15.

[14] AOAC, Official Methods of Analysis. *Association of Official Analytical Chemists*, Washington, DC, 1 (2002) 16th edition.

[15] N.G. Asp, C.G. Johansson, H. Hallmer and M. Siljestrom, Rapid enzymatic assay of insoluble and soluble dietary fibre. *Journal of Agricultural and Food Chemistry*, 31 (1983) 476-482.

[16] D.B. Thompson and J.W. Erdman, Phytic acid determination in soyabeans. *Journal of Food Science*, 47 (1982) 513-517.

[17] M.L. Prince, S. Van Scoyoc and L.G. Buttler, Critical evaluation of the vanillin reaction as an assay for tannin in sorghum-grain. *Journal of Agriculture and Food Chemistry*, 26 (1978) 1214-1216.

[18] W.R. Akeson and M.A. Stahmann, A pepsin pancreatin digest index of protein quality. *Journal of Nutrition*, 83 (1964) 257-261.

[19] J. Luten, H. Crews, A. Flynn, P.V. Dael, P. Kastenmeyer, R. Hurrell, H. Deelstra, L. Shen and S. Fair-weather Trait, Interlaboratory trial on the determination of in vitro dialyzability from food. *Journal of the Science of Food and Agriculture*, 72 (1996) 415-424.

[20] C.R. Llames and J. Fontaine, Determination of amino acids in feeds: collaborative study. *Journal of AOAC International*, 77 (1994) 1362-1402.

[21] S. Liang, Chemical characteristic and fatty acid profile of foxtail millet bran oil. *Journal of American Oil Chemical Society*, 87 (2010) 63-67.

[22] S.B. Elhardallou and A.F. Walker, The water holding capacity of three starchy legumes in the raw, cooked and fibre-rich fraction forms. *Plant Foods for Human Nutrition*, 44(2) (1993) 171-179.

[23] R.A. Anderson, H.F. Conway, V.F. Pfeifer and E.L. Griffin, Roll and extrusion cooking of grain sorghum grits. *Cereal Science Today*, 14 (1969) 372-375.

[24] F.W. Sosulski, E.S. Humbert, K. Bui and J.D. Jones, Functional properties of rapeseed flours, concentrates and isolates. *Journal of Food Science*, 41 (1976) 1349-1352.

[25] C.W. Coffmann and V.V. Garcia, Functional properties and amino acid content of a protein isolate from mung bean flour. *International Journal of Food Science and Technology*, 12 (1977) 473-484.

[26] SPSS for Windows. Version 16.0. SPSS Inc, (2007) Chicago.

[27] B. Menegassi, A.M.R. Pilosof and J.A.G. Areas, Comparison of properties of native and extruded amaranth (*Amaranthus cruentus L.* – BRS Alegria) flour. *LWT- Food Science and Technology*, 44 (2011) 1915-1921.

[28] J.M. Sanz-Penella, M. Wronkowska, M. Soral-Smietana and M. Haros, Effect of whole amaranth flour on bread properties and nutritive value. *LWT-Food Science and Technology*, 50 (2013) 679-685.

[29] K.V.P. Kumar, U. Dharmaraj, S.D. Sakhare and A.A. Inamdar, Preparation of protein and mineral rich fraction from grain amaranth and evaluation of its functional characteristics. *Journal of Cereal Science*, 69 (2016) 358-362.

[30] B. Valcárcel-Yamani and S.C.S. Lannes, Applications of quinoa (*Chenopodium Quinoa Willd.*) and Amaranth (*Amaranthus Spp*.) and their influence in the nutritional value of cereal based foods. *Food and Public Health,* 2 (2012) 265-275.

[31] H. Twinomuhwezi, C.G. Awuchi and M. Racharl Rachael, Comparative study of the proximate composition and functional properties of composite flours of amaranth, rice, millet, and soybean. *American Journal of Food Science and Nutrition*, 6(1) (2020) 6-19.

[32] L.M. Lamothe, S. Srichuwong, B.L. Reuhs and B.R. Hamaker, Quinoa (*Chenopodium quinoa W*.) and amaranth (*Amaranthus caudatus L.)* provide dietary fibre high in pectic substances and xyloglucans. *Food Chemistry*, 167 (2015) 490-495.

[33] P.E. Akin-Idowu, O.T. Ademoyegun, Y.O. Olagunju, A.O. Aduloju and U.G. Adebo, Phytochemical content and antioxidant activity of five grain amaranth species. *American Journal of Food Science and Technology*, 5 (2017) 249-255. S.J. Hur, B.O. Lim, E.A. Decker and J. Mc Clements, In vitro human digestion models for food applications. *Food Chemistry*, 125 (2011) 1-12.

[34] L. Alvarez-Jubete, E.K. Arendt and E. Gallagher, Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients. *Trends in Food Science and Technology*, 21 (2010) 106-113.

[35] M. Mathur, A. Kumari and R. Grewal, Physical and functional properties of different cereals, pulses, millet and oil seeds. *Journal of AgriSearch*, 7(2) (2020) 97-103.

[36] A.C. Nascimento, C. Mota, I. Coelho, S. Gueifão, M. Santos, A.S. Matos, A. Gimenez, M. Lobo, N. Samman, and I. Castanheira, Characterisation of nutrient profile of quinoa (*Chenopodium quinoa*), amaranth (*Amaranthus caudatus*), and purple corn (*Zea mays* L.) consumed in the North of Argentina: Proximates, minerals and trace elements. *Food Chemistry*, 148 (2014) 420-426.

[37] K.I. Kasozi, S. Namubiru, A.A. Safiriyu, H.I. Ninsiima, D. Nakimbugwe, M. Namayanja and M.B. Valladares, Grain amaranth is

associated with improved hepatic and renal calcium metabolism in type 2 diabetes mellitus of male wistar rats. *Evidence-Based Complementary and Alternative Medicine*, 4098942 (2018) 1-10.

[38] Y. Tang, X. Li, P.X. Chen, B. Zhang, R. Liu, M. Hernandez, J. Draves, M.F. Marcone and R. Tsao, Assessing the fatty acid, carotenoid, and tocopherol compositions of amaranth and quinoa seeds grown in Ontario and their overall contribution to nutritional quality. *Journal of Agricultural Food Chemistry*, 64 (2016) 1103-1110.

[39] A. Shukla, N. Srivastava, P. Suneja, S.K. Yadav, Z. Hussain, J.C. Rana and S. Yadav, Untapped amaranth (*Amaranthus spp.*) genetic diversity with potential for nutritional enhancement. *Genetic Resource Crop Evolution*, 65 (2018) 243-253.

[40] WHO, Protein and amino acid requirements in human nutrition (WHO technical report series no. 935). *World Health Organization technical report series*. (2007) Geneva, Switzerland.

[41] S.Y. Gebreil, M.I.K. Ali and E.A.M. Mousa, Utilization of amaranth flour in preparation of high nutritional value bakery products. *Food and Nutrition Sciences*, 11 (2020) 336-354. DOI: 10.4236/fns.2020.115025.

[42] A. Banerji, L. Ananthanarayan and S. Lele, Rheological and nutritional studies of amaranth wheat chapatti (Indian flat bread). *Journal of Food Processing and Preservation*, (2017) 1-8.

[43] S.R. Naik, K.B. Dachana, D. Ramesh and J. Prakash, Nutritional composition and functional properties of KMR 204 and ML 365 varieties of finger millet fractions. *International Journal of Food, Nutrition and Dietetics*, 6 (2018) 13-19.

[44] S.D. Sakhare, A.A. Inamdar, K.V.P. Kumar and U. Dharmaraj, Evaluation of roller milling potential of amaranth grains. *Journal of Cereal Science*, 73 (2017) 55-61.

[45] A.J. Bolontrade, A.A. Scilingo and M.C. Aňón, Amaranth proteins foaming properties: Film rheology and foam stability- part 2. *Colloids and Surfaces B: Biointerfaces*, 141 (2016) 643-650.

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