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Quality Assessment and Evaluation of Amino Acid Profile of Black Wheat Supplemented Noodles

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ABSTRACT

Background: Functional foods are a topic of ongoing industrial use and research. Black colored wheat among the colored wheat grains is being prioritized more in research since it is a source of neutraceutical chemicals such as anthocyanins and rich in essential amino acid content having the potential to offer extra health advantages over those that standard wheat cultivars typically offer. The present study aimed to evaluate the amino acid profile and effect of processing on loss of amino acids in black wheat supplemented noodles.

Methods: During this investigation the noodles were prepared by replacing Netravati wheat flour with black wheat flour in 10 to 100 per cent proportion. On the basis of sensory evaluation the sample BN_6 (noodles containing 60% black wheat flour and 40% whole Netravati wheat flour) was further utilized for analysis of amino acid profile in comparison with control sample. HPLC was used for the quantification of amino acids in flours and noodles prepared by cold extrusion .

Result: Black wheat flour and Black wheat supplemented noodles samples had higher all the essential amino acid content and % EAA score than standards indicating completeness of protein. Minimum losses in amino acid content were observed in black wheat supplemented noodles. The sample BN_6 as selected on the basis of sensory evaluation having 60 per cent incorporated black wheat flour contained highest concentration of Leucine (3.01 g/100 g) followed by Lysine (2.21 g/100 g) and Isoleucine (1.59 g/100 g) of noodles and Threonine (1.52 g/100 g) and Valine (1.46 g/100 g) in notable concentration.

Key words: Black wheat, Essential amino acid score, Noodles, Protein.

Abbreviations: Ala- Alanine, Arg- Arginine, Asp- Aspartic acid, Cys- Cysteine, EAA- Essential Amino Acid, Glu- Glutamic Acid, Gly- Glycine, His- Histidine, Ile- Isoleucine, Leu- Leucine, Lys- Lysine, Met- Methionine, NEAA- Non-Essential Amino Acid, Phy- Phenylalanine, Pro- Proline, Ser- Serine, TAA-Total Amino Acid, TEAA- Total Essential Amino Acid, Thre- Threonine, TNEAA- Total Non-Essential Amino Acid, Try- Tryptophan, Tyr- Tyrosine, Val- Valine.

INTRODUCTION

A healthy population is one of the country's most valuable resources since it may benefit both society and the economy. Colored grain is currently receiving increased attention in study since it is a source of neutraceutical compounds and higher amount of essential amino acids in them. The essential amino acids (EAA); must be taken from food since can't be produced by the human body (Panthee *et al.,* 2006), are necessary for human nutrition and have a substantial impact on a number of biological processes in addition to serving as antioxidants (Zafar *et al.*, 2014). A single essential amino acid deficiency could prevent the production of additional amino acids and the production of other proteins and biological molecules (Jiang *et al*., 2008).

Wheat (*Triticum aestivum*) makes a sizable contribution to the daily caloric requirements of the wheat-eating population. The nutritional value of wheat grains is determined by the protein content and amino acid composition (Joye, 2019). Wheat's amino acid profile is unbalanced and does not include enough EAA, such as Lys, Thr and Met (Jiang *et al*., 2008). The nutritional background of coloured wheat grains, in particular their amino acid content, is currently poorly understood (Tian *et al*., 2018).

Therefore, in order to evaluate the amino acid profile of black wheat flour and noodles derived from, the current study was designed. This would be the first study to document how processing affects losses of amino acids developed noodles high in anthocyanins. Such information could be useful to meet nutritional requirements of people, particularly of countries with wheat as staple diet.

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MATERIALS AND METHODS

Black wheat grains were obtained from local area of Punjab state and traditional wheat variety Netravati as a control sample procured from Seed Research Centre, VNMKV, Parbhani.

Standardization of recipe for black wheat supplemented noodles

For noodles incorporated with Black wheat flour, slight modifications were made to the method provided by Fu (2008) and Inglett *et al.* (2003) with replacement of whole wheat flour with black wheat flour (10 to 100 per cent) were tried to obtain the best acceptable formulation. Noodles made with 100 percent Netravati wheat variety flour was considered as a control. After mixing of all ingredients in the extruder, extrusion was occurred and strands of 2.0 mm thickness of noodles were obtained and subjected for steaming over boiling water for 2 minutes. Further the steamed noodles were dried in a cabinet tray drier at 55-60°C for 3 hours.

Determination of protein

Protein content was determined by Micro-kjeldhalmethod (AOAC, 2000).

Amino acid profile

High Performance Liquid Chromatography profiles used to understand the amino acid profiling in black wheat and noodles prepared (Fabiani, 2002).

Quality parameters of amino acids

The amino acid score (AAS) of each flour sample and black wheat supplemented noodles was calculated using the reference protein values, as provided by the FAO (2013).

$$\% \text{ AAS} = \frac{\text{Amino acid in test protein (g)}}{\text{Amino Acid in reference pattern (g)}} \times 100$$

Essential amino acids score

The essential amino acids score (EAAE) was calculated with reference to the standard recommended by the FAO/WHO (FAO, 1985) and results were expressed with the methodology of Pires *et al*. (2006).

$$\text{EAAE} = \frac{\text{mg of amino acids/g of protein}}{\text{FAO - WHO recommendation (mg of amino acids/g of protein)}}$$

RESULTS AND DISCUSSION

Amino acid profile of black wheat flour

The results in Table 1 revealed that the black wheat flour reported 17 amino acids which are in good agreement with the results of Sharma *et al*. (2022). Out of the 17 amino acids observed both essential (9) and non-essential amino acids(8) contents followed the order: black wheat flour> Netravati wheat flour.

The overall essential amino acid contents in Black wheat flour were at highest concentration than Netravati wheat flour except Lysine which was high in later (2.27 g/100 g of flour). The greatest value was recorded for leucine (22.23 g/100 g of crude protein), followed by lysine (18.90 g/100 g of crude protein). Most of the values obtained for concentrations of all essential amino acids were somewhat higher than those reported previously. According to Wang *et al*. (2019), environmental factors such as humidity, CO_2 concentration, temperature, various biotic and abiotic stresses during crop growth, soil water content, wheat hardness type (hard, soft, or medium), protein content in grains, genetic makeup of wheat varieties and fertilizer use can all affect the amino acid contents of different wheat flours (Zhang *et al*., 2016).

Among the non-essential amino acids, the black wheat flour exhibited high concentrations of all eight non-essential amino acid than Netravati wheat flour. Glutamic acid (4.92 g/100 g) was the most abundant non-essential amino acid in black wheat flour. Next to Glutamic acid, highest concentration of aspartic acid was observed in Black wheat and Netravati wheat flour samples respectively. All other NEAA were at more concentration in Black wheat flour samples as compared to Netravati wheat flour sample. Most of the values obtained for concentrations of all non-essential amino acids were somewhat higher than those reported previously (Sharma *et al.,* 2022).

The black wheat flour had significant concentration of the total amino acid (28.32 g/100 g) and total essential amino acids (12.94 g/100 g) content than Netravati wheat flour (Table 1) and were higher compared to total amino acids reported by Sharma *et al.* (2022) (13.17 g/100 g). The non-essential amino acids (NEAA) as 15.38 g/100 g for black wheat flour and 12.85 g/100 g for Netravati wheat flour. Tian *et al*. (2018) concluded that the black, blue and green coloured wheat flours were more nutrient-dense than white wheat flour owing to higher levels of amino acids.

The values for per cent essential amino acids (%EAA) in Black wheat flour were 46.48 per cent and these were higher as compared to reported values of per cent essential amino acids (%EAA) (30.05%) by Sharma *et al*. (2022). Similar trend was reported for per cent non-essential amino acids (%NEAA) also as reported by Sharma *et al*. (2022). The higher values of protein content in the black wheat flour might be the reason behind higher values of all amino acids contents. The results are in close agreement the values reported by Siddiqi *et al*. (2020); Pepo and Gyori (2007) and Sibian *et al.* (2016) for essential and non-essential amino acids in wheat.

Quality parameters of amino acids

The completeness of a specific protein is predicted using the amino acid score (AAS). Additionally, a score of 100% or above indicates that a protein is complete with respect to that EAA which is equal to or greater than the standard reference pattern recommended by the FAO/WHO. Less than 100 AAS represents incompleteness of protein with respect to a particular EAA. The AAS for all EAAs were >100 in both Black and Netravati wheat flour samples, indicating the completeness of their proteins with respect to these amino acids (Table 2). Different wheat varieties have various AAS, which can be related to the reference protein utilized, species, geographical origin, harvesting season, environmental circumstances and the physiology and

Table 1: Amino acid profile of black wheat flour.

EAA	Flour type				*Amino acid requirement for adults (g/100 g of crude protein)
	Netravati wheat		Black wheat		
	g/100 g of flour	g/100 g of crude protein	g/100 gof flour	g/100 g of crude protein	
Histidine	0.48	4.00	0.61	4.53	1.5
Isoleucine	1.39	11.57	1.73	12.83	3.0
Leucine	2.67	22.23	3.24	24.04	5.9
Lysine	2.27	18.90	2.17	16.10	4.5
Methionine	0.48	4.00	0.56	4.15	1.6
Phenylalanine	0.79	6.58	0.97	7.20	3.0
Threonine	1.36	11.32	1.62	12.02	2.3
Tryptophan	0.39	3.25	0.49	3.64	0.6
Valine	1.33	11.07	1.55	11.50	3.9
Non-essential amino acids					
Alanine	1.06	8.83	1.31	9.72	-
Arginine	0.7	5.83	0.9	6.68	-
Aspartic acid	2.55	21.23	2.97	22.03	-
Glutamic acid	4.03	33.56	4.92	36.50	-
Glycine	0.42	3.50	0.5	3.71	-
Proline	2	16.65	2.28	16.91	-
Serine	1.39	11.57	1.7	12.61	-
Tyrosine	0.7	5.83	0.8	5.93	-
Total amino acid profile					
TAA	24.01	199.92	28.32	210.1	-
TEAA	11.16	92.92	12.94	96.01	-
%EAA	46.48	46.48	45.69	45.70	-
TNEAA	12.85	107	15.38	114.09	-
%NEAA	53.52	53.52	54.31	54.30	-

*Each value is average of three determinations (Source: FAO/WHO/UNU 1985 and 2002).

Table 2: Quality parameters of amino acids: Per cent essential amino acid score.

Amino acids	RP*	Flour type	
		Black wheat	Netravati wheat
Histidine	22	205.91	181.67
Isoleucine	54	237.59	214.33
Leucine	86	279.53	258.51
Lysine	70	230.0	270.01
Met+Cys	57	72.81	70.12
Phy+Tyr	93	77.42	70.73
Threonine	47	255.74	240.93
Tryptophan	17	214.12	191.02
Valine	66	174.24	167.79

Each value is average of three determinations; RP- Reference protein (Chicken egg) (mg/g of crude protein).

genetics of each species, as well as the method used to measure them (Jiang *et al*., 2008).

Effect of black wheat flour incorporation on amino acid profile of noodles

There was steady increase in the concentration of all the essential and non-essential amino acids with increasing Black wheat flour incorporation except for Lysine as it was decreased because of higher concentration in Netravati wheat flour (Table 3). The sample BN_6 as selected on the basis of sensory evaluation having 60 per cent incorporated Black wheat flour contained highest concentration of Leucine (3.01 g/100 g) followed by Lysine (2.21 g/100 g) and Isoleucine (1.59 g/100 g) of noodles respectively. The other essential amino acids such as Threonine (1.52 g/100 g) and Valine (1.46 g/100 g) were also in notable concentration.

The sample BN_6 contained highest concentration of glutamic acid (4.564 g/100 g) followed by aspartic acid (2.802 g/100 g) and proline (2.168 g/100 g) of noodles respectively. The other non-essential amino acids were also in notable concentration. The similar results were obtained by Sharma *et al.* (2022) as concluded in their study as black wheat flour had more contents of all essential and non-essential amino acids except Tyrosine.

The noodles sample BN_6 containing has found to be statistically significant over control noodles sample in all the amino acids concentrations except for Histidine and Methionine, it was at par with control sample.

Essential amino acid profile of selected black wheat supplemented noodles (BN_6)

The results in Table 4 revealed that the sample BN_6 had significant amino score for each amino acid except for

aromatic amino acids (Phenyl Alanine and Tyrosine) which had amino acid score <100 (88.05%) and for Methionine (77.89%) and it might be due to absence of cysteine in black wheat flour and possible lower values of amino acids scores in Black wheat flour itself.

The values for amino acids scores in literature reported previously were >100 for Met + Cys and Phe + Tyr in Black wheat flour and chapatti from it (Sharma *et al*., 2022). Also the values of all the essential amino acids in selected black wheat supplemented noodles were reported significant higher values than the recommended daily allowances values suggested by FAO/WHO. The excellent amino acid profile of prepared black wheat supplemented noodles is an indication of good quality protein.

The black wheat supplemented noodles (BN_6) were fulfilling more than 100 per cent requirement of recommended daily allowances as suggested by FAO/WHO (1985) for all age groups mentioned. Moreover BN_6 sample had all the amino acids to a greater extent exhibiting score>1.00 for children 2-12 years old and for adults too. The EAAE scores above 1.0 are considered to indicate the protein contains essential amino acids in excess of the human requirements WHO/FAO (1985) could be because black wheat has a higher protein content. The amount of essential amino acids requirement decreased as the age group increased, *e.g*., for adults.

Table 3: Effect of Black wheat flour incorporation on essential and non-essential amino acid profile of noodles.

Sample code	His	Ile	Leu	Lys	Met	Phy	Thr	Try	Val
				Essential amino acids (g/100 g of noodles)					
Control	0.48	1.38	2.6	2.25	0.46	0.75	1.35	0.37	1.3
BN_6	0.56	1.59	3.01	2.21	0.53	0.90	1.52	0.45	1.46
SE±	0.059	0.023	0.016	0.013	0.054	0.005	0.015	0.015	0.013
CD at 5%	0.175	0.069	0.046	0.038	0.159	0.014	0.044	0.045	0.038
				Non-essential amino acids (g/100 g of noodles)					
Sample code	Ala	Arg	Asp	Glu	Gly	Pro	Ser	Tyr	
Control	1.04	0.6	2.5	3.99	0.4	1.9	1.3	0.68	
BN_6	1.21	0.82	2.802	4.564	0.468	2.168	1.576	0.76	
SE±	0.030	0.014	0.041	0.095	0.010	0.020	0.018	0.013	
CD at 5%	0.088	0.043	0.122	0.280	0.030	0.060	0.053	0.038	

*Each value is average of three determinations; (the tables were merged and only the values for selected sample were kept for reference).

Table 4: Evaluation of essential amino acid profile of black wheat supplemented Noodles (BN_6) for children and adults in comparison with FAO/WHO (1985).

Amino acids	BN_6		[a]R.P.	[b]RDA	Amino acid	FAO/WHO (1985) Recommendations per day			Score		
	mg/g of noodles	mg/g of crude protein		(mg/kg body wt/day)	score (%)	2-5 years old	10-12 years old	Adults years old	2-5 years old	10-12	Adults
Histidine	5.6	46.9	22	12	213.18	19	19	16	2.47	2.47	2.93
Threonine	15.2	127.4	47	18	271.06	34	28	9	3.75	4.55	14.16
Valine	14.6	122.9	66	29	186.21	35	25	13	3.51	4.92	9.45
Lysine	22.1	185.7	70	35	265.29	58	44	16	3.20	4.22	11.61
Isoleucine	15.9	133.9	54	23	247.96	28	28	13	4.78	4.78	10.30
Leucine	30.1	253.1	86	44	294.30	66	44	19	3.83	5.75	13.32
Tryptophan	4.5	37.8				11	9	5	3.44	4.20	7.56
Phe+Tyr	9.76	81.89	93	30	88.05	63	22	19	1.30	3.72	4.31
Met+Cys	5.3	44.4	57	18	77.89	25	25	17	1.78	1.78	2.61
Total without histidine	116.7	980.7	490	201.8	200.14	-	-	-	-	-	-

*Each value is average of three determinations; Where, a- [a]Reference protein (Chicken egg) (mg/g of crude protein) Source: WHO (1985); b- Source: Nutrient requirement and RDA for infants a report of the expert group of the ICMR 2010 ([b]RDA (mg/Kg body wt/day) for age group more than 2 years (24 months).

Adult daily recommended allowances (DRA) of indispensable amino acids (IAA) and their composition (mg/g of protein) in proteins from black wheat supplemented noodles (BN_6)

Table 5 provides a comparative overview of the amounts of indispensable amino acids in the selected Black wheat supplemented noodles with the WHO/ FAO/UNO adult indispensable amino acid requirements pattern (WHO/FAO/ UNU, 2007). It is revealed that black wheat supplemented noodles were found to have IAA levels higher than the levels suggested by WHO/FAO/UNO pattern for adult human's requirement.

The noodles had lysine content of 185.7 mg/g protein and it contained sulphur amino acids at a level of 44.4 mg/g protein; these were above the suggested levels by WHO/ FAO/UNO. However, total IAAs contents (1033.99 mg IAA/g protein) of Black wheat supplemented noodles exceeded the recommended daily allowance (277 mg IAA/g protein) might be due high content of protein in Black wheat flour. The results obtained in present investigation were higher as compared to reported values in previous literature may be due to varietal difference.

Losses in amino acid contents during noodles making

Losses in Tryptophan was more (5.13 per cent) in control sample and that of Phenyl alanine (2.23 per cent) in black wheat supplemented noodles as reported in Table 6. Ile was the most resistant to reduction in concentration incontrol sample and Leucine in black wheat supplemented noodles. Based upon amino acid losses during noodles preparation, black wheat supplemented noodles samples were found to be better than control samples. Noodles were prepared by cold extrusion process and the contents of amino acid were determined for dry samples therefore the losses in amino acid composition were very little as

Table 5: Adult daily recommended allowances (DRA) of indispensable amino acids (IAA) and their composition (mg/g of protein) in proteins from black wheat supplemented noodles (BN_6).

Essential amino acids	Adult IAA requirement[d]		Black wheat supplemented noodles (BN_6) (mg/g of crude protein)
	mg/kg per day	mg/g protein	
AAA[a]	25	38	81.89
Histidine	10	15	46.9
Isoleucine	20	30	133.9
SAA[b]	15	22	44.4
Valine	26	39	122.9
Leucine	39	59	253.1
Lysine	30	45	185.7
Threonine	15	23	127.4
Tryptophan	4	6	37.8
ΣIAA(%TAA[c])	184	277	1033.99

a- Aromatic amino acids (Phenlylalanine + Tyrosine).

b- Sulfur Amino Acids (Cysteine + Methionine).

c- Sum of all individual amino acids (dispensable plus indispensable) equals to TAA or protein content.

d- WHO/FAO/UNO adult indispensable amino acid requirements pattern (WHO/FAO, 2007).

Table 6: Losses in amino acid contents during noodles making.

EAA	EAA content (mg/g of dry weight)						
	Netravati white wheat			Black wheat			
	Flour	Noodles	% reduction in amino acid content in noodles	Flour	Composite flour*	Noodles (BN_6)	% reduction in amino acid content in noodles
Histidine	4.8	4.7	2.08	6.1	5.58	5.48	1.79
Isoleucine	13.9	13.8	0.72	17.3	15.94	15.9	0.25
Leucine	26.7	26	2.62	32.4	30.12	30.1	0.07
Lysine	22.7	22.5	0.88	21.7	22.1	21.9	0.90
Methionine	4.8	4.6	4.17	5.6	5.28	5.2	1.52
Phenyl alnine	7.9	7.5	5.06	9.7	8.98	8.78	2.23
Threonine	13.6	13.5	0.74	16.2	15.16	15.09	0.46
Tryptophan	3.9	3.7	5.13	4.9	4.5	4.4	2.22
Valine	13.3	13	2.26	15.5	14.62	14.6	0.14

*The amino acid content is as 60 (Black wheat flour): 40 (Netravati white wheat flour) in the proportion flour added for preparation of noodles.

compared to high temperature cooking like steaming or boiling in which amino acids get degraded and oxidized (Adebooye and Singh, 2007) as reported in previous studies. The amino acid losses ranged between (0.07-2.23%) for black wheat supplemented noodles and (0.72-5.13%) for control samples from Netravati wheat flour. Previous studies such as Filip and Vidrih (2015) reported 50.41% reduction in EAA content upon cooking in wheat pasta.

CONCLUSION

Black wheat flour and black wheat supplemented noodles showed greater levels of all essential and non-essential amino acids as well as amino acid scores with percent essential amino acid scores. The black wheat enriched noodles showed the least amount of amino acid content losses, which may have been caused by their higher anthocyanin content than the Netravati white wheat control noodle samples. It has been revealed that anthocyanins have the ability to conceal and protect amino acids and proteins from oxidative alterations and heat. Thus, anthocyanin- rich Black wheat showed improved amino acid stability and might significantly improve the nutritional quality of human meals if employed as food ingredients or as a component of flours. Hence, anthocyanin rich Black wheat could contribute greatly in increasing the nutritional quality of food if included in flours or used as food ingredients.

Conflict of interest

The authors declare that they have no conflict of interest.

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