



A comparative study on the physico-chemical quality indices of whole, commercial and refined wheat flours: Emphasis on essential amino acid index for three major penaeid shrimp species cultured in India

R. JANNATHULLA, K. AMBASANKAR, J. SYAMA DAYAL, B. RAJESH, O. SRAVANTHI AND M. RAJAMOHAMED KALANJIAM

ICAR-Central Institute of Brackishwater Aquaculture, 75, Santhome High Road, RA Puram, Chennai - 600 028
Tamil Nadu, India
e-mail: rjannathulla@gmail.com

ABSTRACT

The physico-chemical characteristics of three different types of refined wheat flours (RWF-1, RWF-2 and RWF-3) were assessed to ascertain its suitability in shrimp feed along with three different types of whole (WWF-1, WWF-2 and WWF-3) and commercial (CWF-1, CWF-2 and CWF-3) wheat flours, and the essential amino acid index (EAAI) was determined based on the dietary requirement of three major penaeid shrimp species cultured in India viz., *Penaeus vannamei*, *Penaeus indicus* and *Penaeus monodon*. Results revealed that the fineness of WWF and RWF was 100% and alcoholic acidity exceeded an ideal level of 0.1% in WWF-2, CWF-3 and RWF-1. RWF had a low ($p < 0.05$) water absorption (51.88-53.93%) and high bulk density (782.92-794.92 g l⁻¹) compared to others. The moisture content was high ($p < 0.05$) in RWF, whereas carbohydrate, protein, lipid and ash were found to be high in WWF. Of all the analysed samples, RWF had a higher ($p < 0.05$) content of wet (28.56-31.82%) and dry (8.90-10.44%) glutes. Among the essential amino acids (EAA), arginine, lysine and methionine did not vary between the samples tested, while others showed significant ($p < 0.05$) difference. EAAI was affected by the species ($p < 0.001$) but not by the flours ($p = 0.173$) and their interactions ($p = 0.468$). Both *P. vannamei* and *P. indicus* had a significantly higher EAAI (0.899 and 0.900, respectively) compared to *P. monodon* (0.862). Regression analysis showed a positive higher correlation between protein and EAA ($r = 0.851$) but EAA and its index were negatively correlated ($r = 0.3464$). Calories from carbohydrates were high in RWF (79.04-81.19%), but the reverse was true for protein and lipid in both WWF and CWF. Results conclude that RWF could be used for shrimp feed production, but ascertaining the processing effect on its utility by shrimps is essential.

Keywords: Chemical composition, Essential amino acid index, Penaeid shrimps, Physical properties, Wheat flour

Introduction

Wheat is a “staple food” as it forms predominant portion of a standard diet with a large fraction of energy and is being consumed routinely. The global production of wheat was about 758.3 million t in 2017 and India emerged as the third largest producer in total global production (USDA, 2018). The states of Uttar Pradesh, Punjab, Haryana, Rajasthan, Madhya Pradesh and Bihar are together contributing >90% of the total Indian production of wheat. After the introduction of formulated feed technology, wheat is widely used for various animal feeds, in particular aquafeed as a major carbohydrate source. FAO (2012) stated that 16.7% of worldwide production is used for feed and the proportion was increased upto 42% in the industrialised countries. Though aquatic organisms, in particular shrimp utilises limited carbohydrate as an energy source, about 20-35% carbohydrate sources are included in the commercial shrimp feed formulation, in which, wheat or wheat-

based materials accounts more than 70% of the total carbohydrates. The USDA (2018) recently estimated that the global consumption of wheat has increased by 13.3 million t which is the highest in recent years primarily due to an increased usage of wheat in commercial feeds. Flour is made from the germ and the endosperm of wheat after removing bran, which is refined and bleached is known as refined wheat flour (RWF). It is extensively used for human consumption, especially in fast foods, breads as well as in varieties of sweets and hence, is also known as “all-purpose flour”. There are more than 900 mills in India producing RWF and its usage is also increasing rapidly in shrimp feed formulation by completely replacing wheat in recent times. The greatest factors affecting the quality of RWF are the freshness of raw materials and its processing. The inside part of wheat kernel which does not contain any fiber, is bleached with benzoic peroxide to obtain white powder and further treated by alloxen to make it very soft. In Indian markets, RWF is available with varying physical

and chemical characteristics. The physical properties, include colour, granularity (fineness), acidity, water absorption and density, and the chemical compositions of moisture, gluten, protein, carbohydrates and ash are important as they indicate quality and suitability. Information on the physical and chemical characteristics of RWF, used to produce processed products for human consumption, is wide (Chandra *et al.*, 2015). However, the specifications of different wheat flours, in particular RWF, that are commonly used for shrimp feed, is not much addressed. Hence, the present study aimed to investigate the physico-chemical quality indices of refined wheat flour (RWF), collected from different parts of India, and compared with whole (WWF) and commercial (CWF) wheat flours along with their essential amino acid index (EAAI) to assess suitability in shrimp feed

Materials and methods

Three different types of raw materials *viz.*, whole wheat flour (WWF), commercial wheat flour (CWF) and refined wheat flour (RWF) were used in the present study. Three samples of whole wheat were collected from the southern and northern parts of Tamil Nadu and Andhra Pradesh, respectively and were ground to fine particles of $<250 \mu$ in a pulveriser. Besides, the most common CWF of three brands available in the local markets in Andhra Pradesh were purchased. Three samples of RWF were directly procured from the distributors, supplying to the shrimp feed manufacturers from different states of India, including Andhra Pradesh, Karnataka and Telangana. All the samples were packed properly in plastic containers and refrigerated at 4°C until use. The physical characteristics [colour, granularity (fineness), alcoholic acidity, water absorption and bulk density] and chemical composition (moisture, glutens, protein, lipids, carbohydrates, ash and acid insoluble ash) of all the test ingredients were analysed.

Physical characteristics

Colour of the ingredients was observed visually and fineness of all the test samples was checked using 85 no. mesh. Alcoholic acidity was determined according to AOAC (1984). Briefly, 50 ml of ethyl alcohol was added to 5 g of sample in a 250 ml stoppered conical flask. The contents were allowed to stand at room temperature for 24 h with occasional swirling. After incubation, they were filtered using Whatman No.1 filter paper and a known volume of filtrate was titrated against 0.05 N sodium hydroxide solution till a pale pink colour was obtained using phenolphthalein as indicator. A blank was treated simultaneously without sample. The method of Sosulski *et al.* (1976) was used to measure the water absorption with slight modifications. Briefly, the sample was immersed in

water in the ratio of 1:10 and allowed to stand at ambient temperature ($27\pm 2^\circ\text{C}$). After 20 min of incubation, the excess water was removed by centrifugation (3000 rpm). Water retention was examined as per cent water retained per 100 g of sample. Bulk density was determined by the method of Wang and Kinsella (1976). A known weight (50 g) of sample was placed in a 100 ml graduated cylinder and packed by gentle tapping of the cylinder on a bench top, more than ten times, from a height of 8-10 cm or till getting a constant volume. The final volume of the sample was measured and the bulk density was expressed as g l^{-1} .

Chemical composition

Proximate composition of ingredients was analysed as per the method of AOAC (1997). The moisture content was determined by drying the samples overnight at 105°C in a hot air oven. Nitrogen content was analysed by the micro Kjeldahl method (Kjeltec™-8100, Tecator™ Line) and the analysed nitrogen was converted into protein by multiplying with a common empirical factor of 6.25. Lipid was estimated using petroleum ether (60 to 80°C) in Soxhlet extraction unit (Socs Plus-SCS6). Total ash content was measured by incinerating samples at 540°C in a muffle furnace for 6 h and was further dissolved in diluted (1:3) acid mixture of HNO_3 and HCl to determine the acid insoluble ash. Carbohydrate was calculated by a difference: $100 - (\% \text{ moisture} + \% \text{ crude protein} + \% \text{ ether extract} + \% \text{ total ash})$ (AOAC, 1997; Jannathulla *et al.*, 2018). The gross energy of test ingredients was measured using a semi-micro oxygen bomb calorimeter (Parr-1425). Both dry and wet gluten was analysed as per AOAC (1984). For this, 100 g of sample was weighed in a plastic tray and was made into a dough by slowly adding water, the dough was spread in the same tray and filled with water. After an hour, the dough was placed on a piece of bolting silk cloth with an aperture of 0.16 mm and washed with a gentle stream of water till water passing through the silk did not give a blue colour with a drop of iodine solution. The cloth was then spread tight on a porcelain plate to facilitate scraping. The residue collected was squeezed to remove excess water and weighed for wet gluten. This was further transferred to a petridish and kept in a hot air oven at $105\pm 1^\circ\text{C}$ overnight to get dry gluten.

Essential amino acid (EAA) profiles were analysed using a pre-column derivatisation HPLC gradient system (Shimadzu Corp, LC-30AD) after hydrolysing the samples with 6 N hydrochloric acid in a sealed tube filled with nitrogen for 22 h at 110°C in an oven (Finlayson, 1964). The acid was dried using a vacuum rotary evaporator (IKA, RE 10 C S84) and the residue was brought into a diluent (0.1 N hydrochloric acid) and then filtered using a $0.2 \mu\text{m}$ membrane syringe filter. The YMC-Triart C18, RRH ($1.8 \mu\text{m}$, $2.1\times 100 \text{ mm}$) column

was used to separate the amino acids after derivatisation with mercaptopropionic acid, O-phthalaldehyde and fluorenylmethoxycarbonyl chloride under gradient elution using phosphate buffer (20 mmol as mobile phase A) and combination of acetonitrile: methanol: water (45:40:15 as mobile phase B) at the flow rate of 0.3 ml min⁻¹. The gradient was changed by increasing mobile phase B concentration at the rate of 11 to 13% at 3 min, 31% at 5 min, 37% at 15 min, 70% at 20 min and 100% at 25 min. Amino acids were quantified by fluorescent detector (RF-20AXS) using amino acid mixer as an external standard (Sigma Aldrich) and norleucine as an internal standard. Tryptophan, being liable to acid hydrolysis, was measured after alkali hydrolysis by spectrophotometric method at 500 nm (Sastry and Tammuru, 1985). The partial oxidation of sulphur containing amino acids like cystine and methionine during acid digestion was prevented by adding 0.1% of phenol (Jajic *et al.*, 2013). Essential amino acid index (EAAI) was calculated based on the amino acid requirements of *Penaeus vannamei*, *Penaeus indicus* and *Penaeus monodon* (Akiyama *et al.*, 1991).

Statistical analysis

Data on physical properties and chemical composition were subjected to one-way analysis of variance (ANOVA). Two-way ANOVA was performed using two different factors *viz.*, wheat flours (WWF, CWF and RWF) and species (*P. vannamei*, *P. indicus* and *P. monodon*) to find difference in EAAI. All the data obtained in the present study were analysed using SPSS version 16.0 and mean comparisons were performed using Tukey's test for significant effect at $p < 0.05$. Prior to statistical evaluation, data were checked for homogeneity of variance after ascertaining the normal distribution pattern. Regression analysis was performed to assess the relationship between protein and EAA as well EAA and its index.

Results and discussion

Physico-chemical properties are the major intrinsic parameters that reflect the interaction between composition, structure and confirmation of the food components, in particular protein and nature of the environment in which they are associated and measured (Kinsella and

Melachouris, 1976). The functional properties of various wheat flours collected from different places are depicted in Table 1. The colour of the ingredients is much important as it influences colour of the end products. The flour colour was determined by visual comparison in our study and revealed that both WWF and CWF were pale brown and RWF was creamish in colour. Keran *et al.* (2009) suggested that colour difference is mainly attributed to the contamination of bran. Though WWF contains all the carotenes which is responsible for creamish colour in RWF, brown colour in WWF was attributed to the presence of bran. Colour of the flour is also influenced by the coarseness or fineness of the material. All the test ingredients were passed through a 85 no. sieve to obtain correct degree of fineness whereas fineness was in the range of 94.66-98.58% in CWF. It was observed that alcoholic acidity was 0.07% in both RWF-2 and RWF-3 and 0.09% in CWF-2, while all other samples had $>0.10\%$. The ideal level of alcoholic acidity was $<0.12\%$ and this level was crossed by CWF-3 and RWF-1 (0.13%) among the analysed samples. Higher alcoholic acidity indicates presence of higher acidity of the germ oil in the test materials, though there is no direct relationship or equivalence between them.

Water absorption was significantly ($p < 0.05$) low in RWF (51.88-53.93%) and ranged between 55.90-61.89% in CWF and 62.44-64.93% in WWF. Chandra and Samsheer (2013) suggested that the higher water absorption would be related to the higher content of hydrophilic constituents, especially polysaccharides. Kuntz (1971) reported that the flour with the higher polar amino acids increased water absorption. Low moisture content of WWF and CWF (Table 2) was also responsible for their enhanced water absorption. This result is corroborated with the findings in African star apple flour (Isah *et al.*, 2013). In contrast to our study, a very high water absorption (140%) was reported in wheat flour earlier (Chandra and Samsheer, 2013). The observed variations between the studies could not only be due to the level of carbohydrates but also related to the degree of protein interactions with water and conformational characteristics (Butt and Batool, 2010). Bulk density was significantly ($p < 0.05$) affected

Table 1. Physical characteristics of whole, commercial and refined wheat flours used in the present study (n=3)

Particulars	Whole wheat flour			Commercial wheat flour			Refined wheat flour (Maida)			SEM (±)	p-value	CV (%)
	WWF-1	WWF-2	WWF-3	CWF-1	CWF-2	CWF-3	RWF-1	RWF-2	RWF-3			
Colour	Pale brown			Pale brown			Creamish			-	-	-
Fineness (%)	100.00 ^a	100.00 ^a	100.00 ^a	94.66 ^d	98.58 ^b	95.66 ^c	100.00 ^a	100.00 ^a	100.00 ^a	0.140	<0.001	0.498
Alcoholic acidity (%)	0.10 ^c	0.11 ^b	0.10 ^c	0.10 ^c	0.09 ^c	0.13 ^a	0.13 ^a	0.07 ^d	0.07 ^d	0.000	<0.001	5.456
Water absorption (%)	62.44 ^{ab}	64.87 ^a	64.93 ^a	60.90 ^b	55.90 ^c	61.89 ^{ab}	51.88 ^d	52.41 ^{cd}	53.93 ^{cd}	2.661	<0.001	3.652
Bulk density (g l ⁻¹)	761.87 ^b	773.10 ^{ab}	762.31 ^b	779.88 ^{ab}	707.89 ^c	773.42 ^{ab}	789.91 ^a	791.92 ^a	782.92 ^{ab}	134.259	<0.001	1.982

Means bearing the same superscripts in a row do not differ significantly ($p > 0.05$)

Table 2. Chemical composition of whole, commercial and refined wheat flour used in the present study (n=3)

Particulars	Whole wheat flour			Commercial wheat flour			Refined wheat flour (Maida)			SEM (±)	p-value	CV (%)
	WWF-1	WWF-2	WWF-3	CWF-1	CWF-2	CWF-3	RWF-1	RWF-2	RWF-3			
Moisture (%)	8.96 ^f	9.03 ^f	9.07 ^f	9.40 ^c	11.99 ^c	10.34 ^d	12.39 ^b	12.26 ^b	12.81 ^a	0.006	<0.001	0.946
Carbohydrates (%) ¹	73.83 ^{de}	73.64 ^e	74.52 ^{bc}	76.01 ^a	72.94 ^f	74.43 ^c	75.03 ^b	74.28 ^{cd}	74.11 ^{cde}	0.057	<0.001	0.423
Wet gluten (%)	25.36 ^e	25.29 ^e	25.45 ^e	22.21 ^f	26.50 ^d	20.81 ^g	28.56 ^c	30.14 ^b	31.82 ^a	0.080	<0.001	1.416
Dry gluten (%)	9.63 ^{bc}	8.89 ^{cd}	9.26 ^{cd}	8.03 ^{ef}	8.79 ^{de}	7.79 ^f	8.90 ^{cd}	10.34 ^{ab}	10.44 ^a	0.113	<0.001	4.851
Protein (%)	14.18 ^a	14.41 ^a	13.49 ^b	12.20 ^d	12.45 ^{cd}	12.77 ^d	11.37 ^e	12.37 ^d	12.78 ^c	0.022	<0.001	1.503
Lipid (%)	1.46 ^a	1.36 ^a	1.38 ^a	1.03 ^b	1.03 ^b	1.04 ^b	0.56 ^c	0.55 ^c	0.68 ^c	0.014	<0.001	15.404
Total ash (%)	1.57 ^a	1.57 ^a	1.54 ^a	1.36 ^b	0.77 ^c	1.42 ^{ab}	0.64 ^{cd}	0.54 ^{de}	0.44 ^e	0.005	<0.001	8.608
Acid insoluble ash (%)	0.02 ^a	0.01 ^a	0.02 ^a	0.01 ^a	0.01 ^a	0.03 ^a	BDL ²	BDL ²	BDL ²	0.000	<0.001	161.635

Means bearing same superscripts in a row do not differ significantly ($p > 0.05$)

¹Calculated by a difference

²Below detectable level

by the ingredient types in the present study and was high in all three RWF samples (782.92-794.92 g l⁻¹), while it was 707.89-779.88 g l⁻¹ in CWF and 761.87-773.10 g l⁻¹ in WWF. The values reported in our study were almost similar to those reported earlier for wheat flour (Chandra *et al.*, 2015; David *et al.*, 2015). High bulk density in RWF can be relatively attributed to the higher content of moisture.

As moisture content is an indicator of storability, determining the same is vital in analysing flour quality. In the present study, the moisture content significantly ($p < 0.05$) varied among the analysed samples and was high in RWF (12.26-12.81%) compared to WWF (8.96-9.07%) and CWF (9.40-11.99%). Masood *et al.* (2004) suggested that the genetic makeup of wheat attributed to the agronomic and climatic condition would be a reason for obtaining a difference in moisture content among the tested samples. Keran *et al.* (2009) stated that the level of moisture should be limited to 14% in whole wheat and 15% in wheat flour to avoid the deterioration of samples that occurs mainly due to the presence of molds, bacteria and other insects, in particular during prolonged storage. Our results are in agreement with the findings of Keran *et al.* (2009) that all the analysed samples had less than 15% moisture. Trajkovic *et al.* (1983) documented that low moisture content did not only increase the storage duration, but was also profitable for the manufacturer. However, wheat and wheat-based products with the moisture content of 12-14% would be ideal for shrimp feed production according to aqua-nutritionists. Wheat and its flour are one of the cost-effective carbohydrate materials and are mainly used as an energy source in shrimp feed. The total carbohydrate content was 73.64-74.52% in WWF, 72.94-76.01% in CWF and 74.11-75.03% in RWF. Though significant ($p < 0.05$) variations were observed statistically in total carbohydrates, not much of difference was noticed among the selected samples.

Wheat flour is used not only as a carbohydrate source but also used as an excellent source of binder in pelleted shrimp feeds. During feed formulation, it is necessary to select suitable ingredients that make shrimp feed to be water stable with good sinking ability as shrimp is a bottom feeder. This could be achieved by a binder (Lim and Cuzon, 1994). Wheat flour with high gluten content is ideally used as a natural binder in shrimp feed, which is mainly responsible for the binding properties of the feed by influencing the elasticity and extensibility characteristics of flour. Wet gluten was found to be high ($p < 0.05$) in RWF (28.56-31.82%) compared to other two categories (WWF and CWF). Suresh (2007) stated that in general, wet gluten is relatively low in Indian wheat than the ideal level for shrimp feed pelleting (32%). But almost a similar level was obtained in RWF-2 and RWF-3 in our study, while it was 28.56% in RWF-1. Of all the analysed samples, CWF-1 and CWF-3 had a significantly ($p < 0.05$) lower content of wet gluten (22.21 and 20.81%, respectively). AACC (2000) reported that a good product of wheat had high protein with about 35% wet gluten and those had 23% wet gluten with low protein was considered as poor in quality. The highest value of dry gluten was observed in RWF-2 and RWF-3 (10.34 and 10.44%, respectively) and the lowest value was noticed in CWF-3 (7.79%). However, an almost similar trend was noticed for dry gluten as in wet gluten among the test samples.

Though wheat flour is a carbohydrate source, its protein quantity is also an important parameter. Among the selected ingredients, all three wheat flours (WWF-1, 2 and 3) had the highest ($p < 0.05$) content of protein (13.49-14.41%) compared to CWF (12.20-12.77%) and RWF (11.37-12.78%). The values were supported by the findings of Ahmad *et al.* (2005). However, a wide variation was reported by Saeid *et al.* (2015) for the CWF available in Bangladesh (8.67-12.47%), who suggested that this could be due to external factors associated with the crop,

in addition to genetic makeup. Brown (1991) stated that soil nitrogen also can influence the protein level. Gupta *et al.* (1992) documented that there are two protein fractions namely; glutenins and gliadins which mainly constitute gluteins. There is no significant difference for lipid content within the category, however, a significant difference ($p < 0.05$) was observed between the samples. A higher lipid content was found in WWF (1.36-1.46%) followed by CWF (1.03-1.04%) and RWF (0.55-0.68%). The lipid level of WWF was found similar to that reported by David *et al.* (2015) in soft wheat flour (1.33%) and was found to be higher for CWF and RWF.

Total ash content was significantly ($p < 0.05$) low in RWF (0.44-0.64%) compared to WWF (1.54-1.57%) and CWF (0.77-1.42%). Keran *et al.* (2009) suggested that the presence of wheat bran could be a possible reason for obtaining higher ash content in both WWF and CWF compared to RWF. Our results corroborated with the findings of Trajkovic *et al.* (1983) who reported a lower ash content for white flour compared to whole wheat with a higher ash content. Variation in milling extraction could also be a reason for obtaining varied levels of total ash content in the present study as reported earlier (NDSU, 2018). In general, wheat kernel contains about 80% of endosperm, which accounts for about 0.35% of ash in total of 1.5-2.0%. This showed that non-endosperm parts had a higher ash content than that of endosperm. Thus, assessing ash content is a sensitive measure to find the amount of non-endosperm parts, in particular bran present in the flour. Acid insoluble ash was below detectable level in all three samples of RWF and was in the range of 0.01-0.03% in both WWF and CWF.

The EAA of all the test samples are presented in Table 3. Among the EAA, leucine was found to be high and tryptophan was the lowest irrespective of the ingredients

selected in the present study. Of all the EAA, arginine, lysine and methionine were not affected, while all other EAA significantly ($p < 0.05$) varied. Similar to protein, most of the EAA were also high in WWF compared to CWF and RWF. Though a significant ($p < 0.05$) difference was noticed in protein within the category of ingredients, EAA did not differ between the three WWFs selected for the present work. An almost similar trend was found for CWF except histidine and isoleucine, whereas EAAs like histidine, leucine, phenylalanine, threonine, tryptophan and valine significantly ($p < 0.05$) differed between all three RWFs.

In general, the quality of protein is assessed based on the amino acid composition, but its utility completely depends on amino acid requirements of the cultured species. Earlier researchers calculated chemical score by considering limiting amino acids and here EAAI was used based on the overall essential amino acid requirements (Akiyama *et al.* 1991). In our study, EAAI was found to be affected by the species ($p < 0.001$) but not by the flours ($p = 0.173$) and their interactions ($p = 0.468$). EAAI ranged between 0.881 and 0.890 in wheat flours irrespective of the species. Both *P. vannamei* and *P. indicus* had a significantly higher EAAI (0.899 and 0.900, respectively) compared to *P. monodon* (0.862). Positive correlation was observed between the proteins and EAA (Fig. 1) and the correlation coefficient was found to be high ($r = 0.851$). Whereas, EAA and its index were negatively correlated with poor correlation coefficients of $r = 0.3464$ (Fig. 2).

Gross energy was significantly ($p < 0.05$) high in WWF (3873.8-3892.1 cal g^{-1}) compared to CWF (3718.7-3827.2 cal g^{-1}) and RWF (3696.9-3750.6 cal g^{-1}). The calories obtained from carbohydrates was found to be high in RWF and in the range of 79.04-81.19%, while it was 78.42-79.44% in CWF and 75.76-76.95% in WWF.

Table 3. Essential amino acids of whole, commercial and refined wheat flour used in the present study (n=3)

Particulars	Whole wheat flour			Commercial wheat flour			Refined wheat flour (Maida)			SEM (\pm)	p-value	CV (%)
	WWF-1	WWF-2	WWF-3	CWF-1	CWF-2	CWF-3	RWF-1	RWF-2	RWF-3			
Arginine	0.67 ^a	0.67 ^a	0.67 ^a	0.54 ^a	0.60 ^a	0.60 ^a	0.54 ^a	0.41 ^a	0.61 ^a	0.006	0.087	16.969
Histidine	0.34 ^a	0.32 ^{ab}	0.34 ^a	0.28 ^{de}	0.27 ^e	0.31 ^{bc}	0.26 ^e	0.30 ^{cd}	0.30 ^{bcd}	0.001	<0.001	4.633
Isoleucine	0.51 ^a	0.50 ^a	0.48 ^{ab}	0.42 ^{de}	0.40 ^{de}	0.46 ^{bc}	0.40 ^e	0.43 ^{cd}	0.45 ^{bc}	0.001	<0.001	4.093
Leucine	1.03 ^a	1.01 ^a	0.95 ^a	0.74 ^e	0.81 ^{bc}	0.82 ^{bc}	0.75 ^{bc}	0.81 ^{bc}	0.83 ^b	0.002	<0.001	6.205
Lysine	0.42 ^a	0.42 ^a	0.43 ^a	0.35 ^a	0.36 ^a	0.35 ^a	0.36 ^a	1.45 ^a	0.39 ^a	0.223	0.486	123.535
Methionine	0.22 ^a	0.21 ^a	0.23 ^a	0.20 ^a	0.22 ^a	0.21 ^a	0.21 ^a	0.22 ^a	0.21 ^a	0.001	0.771	9.900
Phenylalanine	0.64 ^a	0.63 ^a	0.63 ^a	0.54 ^{cd}	0.56 ^{bc}	0.54 ^{cd}	0.52 ^d	0.59 ^b	0.58 ^{bc}	0.001	<0.001	3.725
Threonine	0.42 ^a	0.42 ^a	0.42 ^a	0.36 ^{bc}	0.35 ^{cd}	0.35 ^{cd}	0.32 ^d	0.38 ^b	0.38 ^b	0.001	<0.001	4.183
Tryptophan	0.16 ^{ab}	0.17 ^a	0.17 ^a	0.14 ^{bc}	0.15 ^{ab}	0.16 ^{ab}	0.12 ^e	0.15 ^{ab}	0.15 ^{ab}	0.002	0.005	7.845
Valine	0.62 ^a	0.61 ^{ab}	0.60 ^{ab}	0.50 ^e	0.54 ^d	0.55 ^{cd}	0.50 ^e	0.55 ^{cd}	0.58 ^{bc}	0.003	<0.001	3.716

Means bearing the same superscripts in a row do not differ significantly ($p > 0.05$)

Table 4. Essential amino acids index (EAAI) of whole, commercial and refined wheat flour for *P. vannamei*, *P. indicus* and *P. monodon* (n=3)

Particulars	EAAI
Wheat flour	
WWF	0.881 ^a
CWF	0.890 ^a
RWF	0.890 ^a
Species	
<i>P. vannamei</i>	0.899 ^a
<i>P. indicus</i>	0.900 ^a
<i>P. monodon</i>	0.862 ^b
Interactions	
WWF x <i>P. vannamei</i>	0.892 ^a
WWF x <i>P. indicus</i>	0.889 ^a
WWF x <i>P. monodon</i>	0.861 ^a
CWF x <i>P. vannamei</i>	0.901 ^a
CWF x <i>P. indicus</i>	0.901 ^a
CWF x <i>P. monodon</i>	0.869 ^a
RWF x <i>P. vannamei</i>	0.905 ^a
RWF x <i>P. indicus</i>	0.908 ^a
RWF x <i>P. monodon</i>	
p values	
Wheat flours (A)	0.173
Species (B)	<0.001
A x B	0.468
Pooled SEM (\pm)	0.001
CV (%)	1.293

Means bearing same superscript letters in a column within the main effects and interactions between the categories do not differ significantly ($p>0.05$)

Table 5. Gross energy and calories (cal g^{-1}) obtained from each nutrients of whole, commercial and refined wheat flour used in the present study (n=3)

Particulars	Whole wheat flour			Commercial wheat flour			Refined wheat flour (Maida)			SEM (\pm)	p-value	CV (%)
	WWF-1	WWF-2	WWF-3	CWF-1	CWF-2	CWF-3	RWF-1	RWF-2	RWF-3			
Gross energy	3892.7 ^a	3888.1 ^{ab}	3873.8 ^b	3827.2 ^c	3718.7 ^f	3796.9 ^d	3696.9 ^e	3722.0 ^f	3750.6 ^e	56.66	<0.001	0.26
Calories from carbohydrates	2953.2 ^{de}	2945.6 ^c	2980.8 ^{bc}	3040.2 ^a	2917.8 ^f	2977.4 ^c	3001.4 ^b	2971.4 ^{cd}	2964.5 ^{cde}	91.09	<0.001	0.42
Calories from protein	800.9 ^a	813.9 ^a	762.0 ^b	689.3 ^d	703.2 ^{cd}	721.5 ^c	642.2 ^e	698.9 ^d	721.8 ^c	69.18	<0.001	1.50
Calories from lipid	138.6 ^a	128.5 ^a	131.0 ^a	97.6 ^b	97.6 ^b	97.9 ^b	53.2 ^c	51.6 ^c	64.2 ^c	125.25	<0.001	15.40

Means bearing same superscripts in a row do not differ significantly ($p>0.05$)

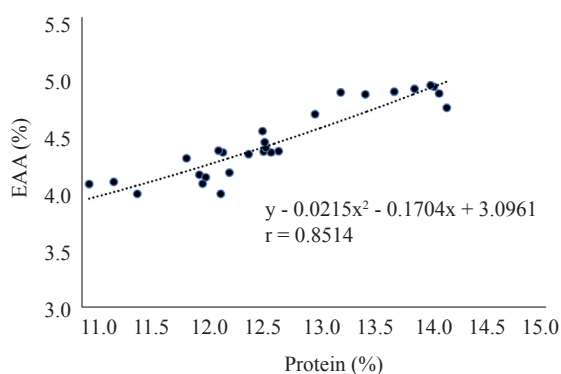


Fig. 1. Regression analysis between the protein and essential amino acid (EAA) of whole, commercial and refined wheat flours

However, the reverse trend was true for the calories obtained from protein and lipid (Table 5).

It can be concluded that refined wheat flour is available with varied functional properties and chemical composition in India. This study recommends that refined wheat flour also could be used for shrimp feed production as they almost meet the specific requirements as in whole and commercial wheat flours. However, further studies need to be undertaken to ascertain the effect of processed refined wheat flour incorporated shrimp feeds on the growth of shrimps.

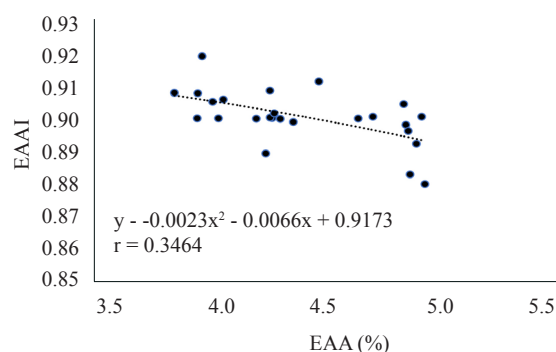


Fig. 2. Regression analysis between the essential amino acid (EAA) and its index (EAAI) of whole, commercial and refined wheat flours

Acknowledgements

The assistance rendered by Y. Thirupalu, L. Santhosh and P. Munikumar in the present research work is duly acknowledged.

References

- AACC 2000. *Approved methods*, 10th edn. American Association of Cereal Chemists, St. Paul, Minnesota, USA.
- Ahmad, I., Ahmad, N., Kausar, T. and Ashraf, M. 2005. Effect of maltogenic amylase on the shelf life of bread, *Pak. J. Food Sci.*, 15: 15-19.
- Akiyama, D. M., Dominy, W. G. and Lawrence, A. L. 1991. Penaeid shrimp nutrition for the commercial feed industry.

- In: Fast, A. L. and Lester, L. J. (Eds.), *Marine shrimp culture: Principles and practices*. Elsevier Science Publishers BV, Amsterdam, The Netherlands. p. 535-568.
- AOAC 1984. *Official methods of analysis*, 14th edn. Association of Official Analytical Chemists, Benjamin Franklin Station, Washington DC, USA.
- AOAC 1997. *Official methods of analysis*, 18th edn. Association of Analytical Chemists, Benjamin Franklin Station, Washington DC, USA.
- Brown, K. H. 1991. The importance of dietary quality versus quantity for weanlings in the developed countries, A framework for discussion. *Food Nutr. Bull.*, 13: 86-93. <https://doi.org/10.1177/156482659101300219>.
- Butt, M. S. and Batool, R. 2010. Nutritional and functional properties of some promising legume protein isolates. *Pak. J. Nutr.*, 9: 373-379. <https://doi.org/10.3923/pjn.2010.373.379>.
- Chandra, S. and Shamsher 2013. Assessment of functional properties of different flour. *Afr. J. Agric. Res.*, 8: 4849-4852. <https://doi.org/10.5897/AJAR2013.6905>.
- Chandra, S., Singh, S. and Kumari, D. 2015. Evaluation of functional properties of composite flours and sensorial attributes of composite flour biscuits. *J. Food Sci. Technol.*, 52: 3681-3688. <https://doi.org/10.1007/s13197-014-1427-2>.
- David, O., Arthur, E., Kwadwo, S. O., Badu, E. and Sakyi, P. 2015. Proximate composition and some functional properties of soft wheat flour. *Int. J. Inn. Res. Sci. Eng. Technol.*, 4: 753-758.
- FAO 2012. *The state of the world fisheries and aquaculture*, Food and Agricultural Organisation, Rome, Italy.
- Finlayson, A. J. 1964. Amino acid recovering in the analysis of some feed samples. *Can. J. Plant Sci.*, 45: 184-188.
- Gupta, R. B., Batey, I. L. and MacRitchie, F. 1992. Relationships between protein composition and functional properties of wheat flours. *Cereal Chem.*, 69: 125-131.
- Isah, A. P., Danladi, Y. and Ejike, O. J. 2013. Proximate composition and some functional properties of flour from the kernel of African star apple (*Chrysophyllum albidum*). *Int. J. Agric. Pol. Res.*, 1: 62-66.
- Jajic, I., Krstovic, S., Glamocis, D., Jaksis, S. and Abramovic, B. 2013. Validation of an HPLC method for the determination of amino acids in feed. *J. Serb. Chem. Soc.*, 78: 839-850. <https://doi.org/10.2298/JSC120712144J>.
- Jannathulla, R., Dayal, J. S., Ambasankar, K., Eugene, A. C. and Muralidhar, M. 2018. Fungus, *Aspergillus niger*; fermented groundnut oil cake as a fishmeal alternative in the diet of *Penaeus vannamei*. *Aquac. Res.*, 49: 2891-2902.
- Jobling, M. 1983. Influence of body weight and temperature on growth rates of Arctic charr, *Salvelinus alpinus* (L.). *J. Fish Biol.*, 22: 471-475. <https://doi.org/10.1111/j.1095-8649.1983.tb04767.x>.
- Keran, H., Salkic, M., Odobasic, A., Jasic, M., Ahmetovic, N. and Sestan, I. 2009. The importance of determination of some physical-chemical properties of wheat and flour. *Agric. Con. Sci.*, 74: 197-200.
- Kinsella, J. E. and Melachouris, N. 1976. Functional properties of proteins in foods: a survey. *Cr. Rev. Food Sci. Nutr.*, 7: 219-280. <https://doi.org/10.1080/10408397609527208>.
- Kuntz Jr. I. D. 1971. Hydration of macromolecules. III. Hydration of polypeptides. *J. Am. Chem. Soc.*, 93: 514-516. <https://doi.org/10.1021/ja00731a036>.
- Lim, C. and Cuzon, G. 1994. Water stability of shrimp pellet: A review. *Asian Fish. Sci.*, 7: 115-126.
- Masood, S. B., Muhammad, N., Saeed, A. and Kamran, S. 2004. Effect of moisture and packaging on the shelf life of wheat flour. *Int. J. Food Safety*, 4: 1-6.
- NDSU 2018. *Water quality and carbohydrate research*. North Dakota State University. <https://www.ndsu.edu/faculty/simsek/wheat/flour.html>.
- Saeid, A., Hoque, S., Kumar, U., Das, M., Muhammad, N., Rahman, M. M. and Ahmed, M. 2015. Comparative studies on nutritional quality of commercial wheat flour in Bangladesh. *Bangl. J. Sci. Ind. Res.*, 50: 181-188.
- Sastry, C. S. P. and Tammuru, M. K. 1985. Spectrophotometric determination of tryptophan in protein. *J. Food Sci. Technol.*, 22: 146-147.
- Sosulski, F., Humbert, E. S., Bui, K. and Jones, J. D. 1976. Functional properties of rapeseed flours, concentrates and isolates. *J. Food Sci.*, 41: 1349-1352. <https://doi.org/10.1111/j.1365-2621.1976.tb01168.x>.
- Trajkovic, J. E. A., Baras, J., Miric, M. and Siler, S. 1983. *Food analysis*. 1st edn. Faculty of Technology and Metallurgy, Beograd. p 359-378.
- USDA 2018. United States Department of Agriculture. <http://www.indexmundi.com/agriculture/?commodity=soybeanmeal&graph=production> (Accessed 24 January 2019).
- Wang, J. C. and Kinsella, J. E. 1976. Functional properties of novel proteins: Alfalfa leaf protein. *J. Food Sci.*, 41: 286-292. <https://doi.org/10.1111/j.1365-2621.1976.tb00602.x>.