Effect of Incorporating Fish Protein Hydrolysate into Pasta: Impact on Protein Digestibility, Starch Digestibility, Amino Acid, and Fatty Acid Profiles

ABSTRACT

The incorporation of fish protein hydrolysate (FPH) with the concentration of 5, 10, 15 and 20% prepared from fish waste using 1% pepsin enzyme was found beneficial in semolina-based pasta. The *In-vitro* protein and starch digestibility of the FPH incorporated pasta were studied. Besides, amino acid and fatty acid composition of the pasta were also evaluated. The protein digestibility of the pasta was in the range of 80.32% to 83.76% and digestibility curve was generated by plotting the pH and time. The value of sugar released at 20 min, 60 min and 120 min was recorded and a graph was developed showing readily digestible starch (RDS) and slowly digestible starch (SDS). The pasta's diverse amino acid profile, including 8 essentials and 9 non-essentials, alongside saturated fats at 39.93%-44.65% and PUFA at 12.49%-16.27%, collectively define its nutritional quality.

Keywords: Fish protein hydrolysate, Pasta, In-vitro protein and starch digestibility, Amino acid, Fatty acid

1. INTRODUCTION

The quest for developing pasta products with improved nutrition has directed to explore an alternative protein source into conventional food matrices. Pasta has long shelf life (Ribeiro et al., 2021), high glycaemic index (Brennan et al., 2004), deficient in the essential amino acids such as lysine and threonine (Gopalakrishnan et al., 2011) and fatty acids (Ainsa et al., 2021). Amino and fatty acids support body tissue growth and maintenance (Laleg et al., 2019). Pasta, fortified with various animal and plant protein sources such as eggs, fava bean flour, meat, barley flour, shrimp powder, amaranth seed flour, almond flour, quinoa and salmon powder (Desai et al., 2019b). Every year, 7.3 million

tons of fish processing waste rich in amino acids and fatty acids (including viscera, heads, frames, cut-offs, bone, and skin) are discarded (Pierre et al. 2012). Enzymatic hydrolysis of fish proteins has been employed for converting fish waste into valuable products for the pharmaceutical and food industries. Fish protein hydrolysate (FPH), high in protein, essential amino acids, fatty acids and bioactive peptides offers a way to reduce the nutritionally dense fish waste. FPH contain reduced di- and tri-peptides, improves protein digestibility and functional properties of food product (Kristinsson and Rasco 2000). The addition of different protein sources, pangas protein isolates (PPI) (Singh et al., 2021), soy protein isolate with egg white protein (Rachman et al., 2020), and cod (*Pseudophycisbachus*) protein (Desai et al., 2021) to pasta alter the protein digestibility and starch digestibility as compared to control. Protein digestibility, a critical factor, influence the bioavailability and utilization of dietary proteins. Cereal-based pasta contains less protein and digestibility compared to animal protein-based pasta; also, the absence of amino acids (threonine, methionine, and lysine) and specific fatty acids makes them comparatively less nutritious (Carbonaro et al., 2012). Therefore, enriching food products with a high nutritional profile extends the means to enhance protein digestibility while reducing starch digestibility (Li et al., 2014), and additionally, FPH contains beneficial fatty acids (omega-3 polyunsaturated fatty acids - PUFAs) provides nutritional advantage to consumers. PUFAs provide various health benefits, such as cardiovascular health and anti-inflammatory properties. Pasta enriched with sea-bass byproduct (Ainsa et al., 2021) and salmon protein (Desai et al., 2020) showed high level of mono- and poly-unsaturated fatty acids profile. Bread prepared with 5%, 10% and 15% cod protein powder enhanced protein digestibility and reduced predictive glycaemicindex (Desai et al., 2021). Modifying carbohydrate and protein content in cereal food impacts glycaemic index and digestibility, enhancing product quality, as seen with pasta fortified with cod (*Pseudophycisbachus*) & salmon (*Oncorhynchus tschawytscha*) powder (Desai et al., 2021; Desai et al., 2018a) and bread fortified with cobia (*Rachycentron canadum*) powder (Fagundes et al., 2018). Incorporating FPH into pasta presents an opportunity to enhance protein digestibility, starch digestibility, and improve the amino acid and fatty acid profiles of the final product. Therefore, the aim of present study was to prepare a pasta from fish protein hydrolysate and evaluate for its nutritional characteristics such as protein digestibility, starch digestibility, amino acid and fatty acid profile.

2. MATERIAL AND METHODS

2.1 Raw materials

Semolina (Semola di granoduro, durum wheat semolina, Corato (BA) – Italy], purchased from Amazon.in (online), was used for the preparation of pasta. The fish waste was collected

from Mirkarwada Fish Landing Centre and transported in chilled condition to the laboratory of Department of Fish Processing Technology and Microbiology, College of Fisheries, Ratnagiri, Maharashtra, India.

2.2 FPH preparation from fish waste

FPH was prepared with 1% pepsin (10,000 U/mg, Himedia, Maharashtra, India) enzyme in a substrate made from homogenization of fish waste and distilled water in a 1:2 ratio (Tejpal et al., 2021). The homogenate was adjusted to pH 2.5 using 2M HCl prior to the addition of enzyme and incubated for 5 min at 37□ to reach equilibrium temperature. The hydrolysis was performed at 37□ for 3 hours and terminated by heating in boiling water for 15 minutes (Bio Techno Lab, Mumbai, India). Then, the mixture was cooled to room temperature, adjusted to pH 7 with 2 M NaOH, filtered through muslin cloth, and filtrate was centrifuged (Hettich Zentrifugen, D-78532, Germany) for 15 min at 10,000 rpm. The supernatant obtained was dried using a tray in cabinet dryer (Fourtech, Techno industries, 1000 rpm, 230 volt, India) at 55□ for 6 h. The obtained hydrolysate was packed airtight and stored at -20□ for further process.

2.3 Pasta preparation

Pasta was freshly prepared by incorporating FPH in five different concentrations with semolina in the ratio of 0:100; 5:95: 10:90; 15:85 and 20:80 (Desai et al., 2018a) using pasta making machine (Kent, RO systems Ltd., U.P., India) with 50 holes die measuring 2.25 mm diameter. Ingredients were added to the machine and mixed for 20 minutes before water was incorporated. Then, the machine was switched to the extrusion function. The long strands of uniform thickness (approximately 3.5 mm) were collected on a tray and cut at around 10 cm length using a stainless-steel knife. For each batch of pasta preparation, 500 g of dry ingredients and 190 ml of RO water (room temperature) were added. The pasta samples were packed in zip lock bags, stored at -18□, and thawed for 10 minutes at room temperature before analysis.

2.4 *In-vitro* protein digestibility of the pasta

Each FPH-enriched pasta sample was analysed for in vitro protein digestibility using different enzymes (Himedia, Maharashtra, India) and optimal pH conditions (Hsu et al., 1977). A 50 ml protein suspension (6.25 mg of protein/ml) was prepared in distilled water, adjusted to pH 8 with 0.1 N HCl/0.1 N NaOH, and placed on a magnetic heating stirring block at 37□. An enzyme solution containing 1.6 mg/ml trypsin (2000 U/g), 3.1 mg/ml chymotrypsin (2500

U/mg), and 1.3 mg/ml peptidase (Pepsin - 10,000 U/mg) was maintained in an ice bath and adjusted to pH 8.0 using 0.1 N HCl/0.1 N NaOH. 5 ml of the enzyme solution was added to the protein suspension maintained at 37□. The decrease in pH was recorded after adding the enzymatic solution for every 10 minutes using a digital pH meter. The protein digestibility percent (Y) was calculated using the following equation:

$$Y = 210.46 – 18.10 X$$

Where: X = change in pH after 10 min

2.5 *In-vitro* starch digestibility of the pasta

The *In-vitro* carbohydrate digestibility of the pasta samples was performed (Desai et al., 2019), measuring the amount of free reducing sugars released during the enzymatic hydrolysis. The pasta was initially cooked in 600 ml of boiling tap water for the optimum time, then cut into 2-5 mm pieces with a knife. 2.5 g of samples were suspended in 30 ml RO water and placed on a pre-heated 15 place magnetic heated stirring block and maintained at 37□ with constant stirring. The in-vitro stomach digestion began with the addition of 0.8 ml 1 M HCl and 1 ml of 10% pepsin solution in 0.5 M HCl, followed by incubation for 30 min at 37□ with stirring. At time 0, 1 ml aliquots of each sample were taken and mixed with 4 ml ethanol. To prevent end product inhibition of pancreatic α-amylase, 0.1 ml amyloglucosidase (Sigma Aldrich, St Louis USA) was added to the digestion flask. Small intestine digestion commenced with the addition of 5 ml of 2.5% pancreatin (Sigma Aldrich, St Louis USA) in 0.1 M sodium maleate buffer pH 6, stirred constantly at 37□ for 120 min, with aliquots withdrawn after 20, 60, and 120 min and mixed with 4 ml ethanol. The samples were stored for reducing sugar analysis at 4□ using 3,5-dinitrosalicylic acid (DNS). For reducing sugar estimation, sample aliquots were centrifuged for 10 min at 1000 g. Then, 0.05 ml of each sample aliquot from each replicate, along with appropriate controls, were transferred to clean, dry test tubes. A 0.25 ml enzyme solution comprising 1% invertase and 1% amyloglucosidase was added to each tube, followed by incubation for 20 min at room temperature. Subsequently, 0.75 ml DNS reagent was added to each tube, which were then covered, heated for 10 min in a boiling water bath, cooled, and mixed with 4 ml RO water. The absorbance was measured at 530 nm after adjusting the spectrophotometer to zero with RO water as the blank. Reducing sugar content was calculated as mg/g sample and plotted against time, with area under the curve (AUC) calculated by dividing the graph into trapezoids.

2.6 Quantification of total amino acids of the FPH enriched pasta

The total amino acid content of FPH (80.55% protein) (obtained from pepsin treated fish waste) was analysed using high performance liquid chromatography (HPLC) (Agilent Technologies, Palo Alto, California, USA), RP-HPLC (HP-Agilent 1,100 model, Agilent Technologies) technique of Islam et al. (2021). To analyse total tryptophan, 100 mg of protein hydrolysate was combined with 8 ml of 5 mol/L NaOH and heated at 120□ for 22 hours under nitrogen gas. Subsequently, it was neutralized with 6.67 ml of 6 M HCl. Additionally, other amino acids were determined using the same sample amount, which was hydrolysed with 8 ml of 6 mol/L HCl under nitrogen gas, incubated in an oven under identical time and temperature conditions, and neutralized with 4.8 ml of 10M NaOH. For free amino acids evaluation, 1,000 mg protein hydrolysate was taken and diluted with 25 ml of 5% TCA and stranded for 1-2 h and centrifuged for 10 min at 10,000 g. Finally, 1 µl of solutions were injected into HPLC analytical column of 250 × 4.6 mm I.D, 5 µm particle size. The study was made by reverse-phase HPLC assembly system at 338 nm detection, 1.0 ml/min flow rate at 40□ column temperature. Mobile phase A was 7.35 mM/L C2H3NaO2/tri-ethylamine/tetrahydrofuran (500:0.12:2.5, v/v/v) and adjusted to pH 7.20 ± 0.05 with CH3COOH while mobile phase B (pH 7.20 ± 0.05) was 7.35 mM/L C2H3NaO2/CH3OH/acetonitrile (1:2:2, v/v/v). the results were expressed as g/100g.

2.7 Fatty acids profiling of the FPH enriched pasta

Lipids from fish protein hydrolysate and pasta samples were detected with heptane solvent and analysed in gas chromatography coupled with a flame ionisation detector (FID) (Perkin Elmer, Walthan, MA, USA). A VARIAN gas chromatograph model CP 7420 equipped with CP-Sil (100 m long, 025 mm internal diameter and 0.2 µm film thickness) fused silica capillary column and FID was used to separate fatty acid methyl esters. The sample (1 µl) was injected and the temperature injector and detector were set 250□. The ramp rate of the column temperature was 13□/min and 4□/min until 175□ and 215□ respectively which were held at 27 and 35 min respectively. The column temperature was started at 45□. After the oven temperature attained 250□, it was kept for 5 min. The total fatty acid content was detected and expressed in percentage. To establish the correction factors of the fatty acids, a standard solution (68 D) was used to transform the percentage peaks by weight (mg/g of total fatty acids). In this process, helium gas used as the carrier gas with a flow rate of 16.7 cm/s. The methyl ester was quantified using star 6.0 software through the integration of the peak area (Palmquist and Jenkins, 2003).

2.8Statistical analysis

All experiments were conducted in triplicate (n=3) and the data are presented as mean ± SD unless specified otherwise. The results were analysed using one-way ANOVA, and significant differences ($P < 0.05$) were determined using Tukey's comparison test (Snedecor and Cochran, 1967).

3. RESULTS AND DISCUSSION

3.1 Protein content and *In-vitro* protein digestibility of the pasta

The protein digestibility of control and enriched pasta with different FPH concentration showed in the range of 80.32±0.1% to 83.76±0.1% (Table 1, digestibility curve shown in Fig. 1). Protein quality relies on chemical composition, digestibility, and compounds affecting amino acid utilization, determining the nutritional characteristics of food (Lorusso et al., 2017). Protein digestibility is crucial for protein intake. Key factors affecting digestion and absorption include phenolic compounds, anti-nutritional factors, protein inhibitors, and processing methods (Gilani et al., 2012). A study found fish protein to be more digestible than plant protein. The author explains that food protein digestibility involves enzymatic hydrolysis into amino acids or smaller peptides for intestinal absorption (Greco et al., 2017).
In the present study, the protein digestibility was found decreasing ($P > 0.05$) with increased level of FPH incorporation in pasta as compared to control pasta. The control pasta was shown 83.76% protein digestibility and 82.43%, 81.58%, 81.16%, 80.32% for 5%, 10%, 15% and 20% FPH level respectively (Table 1). Similarly, Ramya et al. (2015) observed a decreasing trend in protein digestibility with the influence of freeze-dried shrimp powder in pasta processing using Indian durum wheat, where protein digestibility was 93% for control pasta, and 72%, 90%, and 96% for pasta fortified with 2.5%, 5%, and 10% dried shrimp powder, respectively. Also, De Marco et al. (2014) found decreasing trend in protein digestibility (80.88 to 55.45%) of spirulina fortified pasta with the increased level (5 to 20%) of spirulina in the pasta which was due to the presence of phenolic compounds in the spirulina. Polysaccharides and phenolic compounds are the factors responsible for protein digestion in the food matrix where the interaction between protein and phenolic compounds results to the decrease in the digestibility. Proteins and oxidized phenolic compounds create insoluble complexes, disrupting proteolytic activity and interfering protein utilization (Labuckas et al., 2008). FPH in the pasta, increase the protein content. However, there was no significant difference between the uncooked and cooked pasta. This indicates no protein loss during the cooking process. A similar result was observed by Desai et al. (2018b) in

pasta enriched with red cod fish powder and De Marco et al. (2014) in pasta fortified with spirulina.

In the protein digestibility curve, the pH of the FPH enriched pasta using enzymes (trypsin, chymotrypsin, protease) decreased significantly ($P < 0.05$) (Fig. 1). Protein digestion releases amino acid and peptides, lowering the pH of surrounding medium. Enzyme addition to the protein solution releases carbonyl (-COO-) and amino (-NH3+) groups. Protons (H+) are liberated at neutral pH by deionizing the free amino groups in water and resulted decreased pH of the solution. Additionally, phenolic compounds in the pasta can oxidize with peptide amino groups, forming quinone complexes and protein cross-links. Further, the amino and sulfhydryl groups of proteins react with quinines lowered the protein digestibility (Prodpran et al., 2012).

Table 1: Protein content, In-vitro protein digestibility and protein availability of pasta fortified with FPH

Sample	PC in dry raw pasta (%)	PC in dry cooked pasta (%)	PD (%)	Significance between PC of uncooked and cooked pasta	PA dry pasta (%)
CO	14.02 ± 0.01 [a]	14.18 ± 0.02 [a]	83.76 ± 0.1 [a]	* P < 0.05	12.93 ± 0.005 [a]
5FPH	18.95 ± 0.02 [b]	18.85 ± 0.05 [b]	82.43 ± 0.1 [b]	* P < 0.05	17.14 ± 0.03 [b]
10FPH	22.08 ± 0.06 [c]	22.64 ± 0.04 [c]	81.58 ± 0.3 [c]	* P < 0.05	20.57 ± 0.005 [c]
15FPH	26.72 ± 0.03 [d]	26.92 ± 0.01 [d]	81.16 ± 0.2 [c]	* P < 0.05	25.13 ± 0.005 [d]
20FPH	32.23 ± 0.04 [e]	31.33 ± 0.05 [e]	80.32 ± 0.1 [d]	* P < 0.05	29.28 ± 0.02 [e]

PC- protein content, PD - *In-vitro* protein digestibility, PA- protein availability. CO- control pasta, 5FPH, 10FPH, 15FPH and 20FPH: pasta prepared with 5, 10, 15 and 20g of fish protein hydrolysate /10g of semolina flour respectively. Results in the table represent the mean of triplicate measurements. Mean ± standard deviation (n = 3). Values within a group column followed by the same superscript letter are significantly different from each other ($P < 0.05$) and * indicate not significant ($P < 0.05$). according to Tukey's test

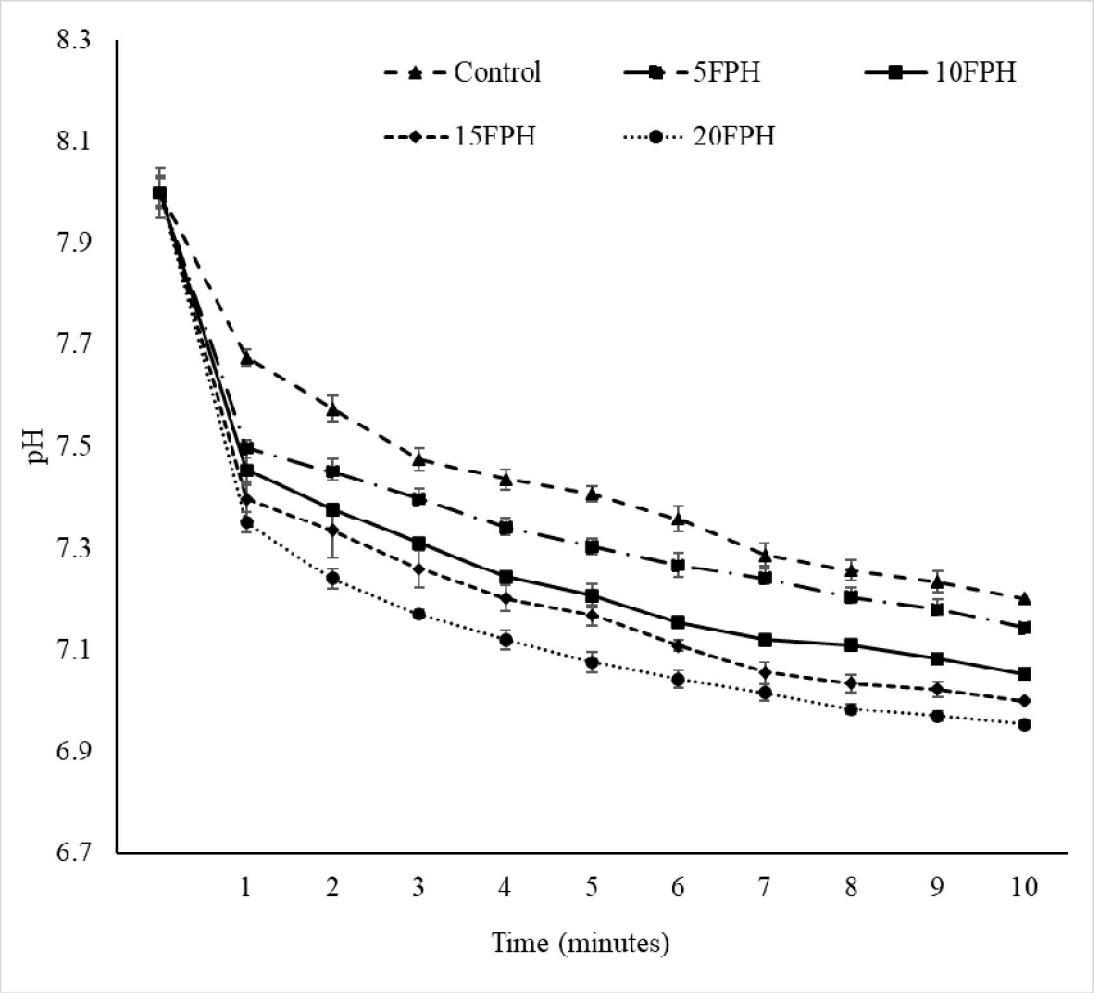

Fig. 1: The pH vs time curves obtained by different concentration of FPH incorporated pasta incubated with multi-enzymes (trypsin, chymotrypsin and protease)

3.2 In-vitro starch digestibility of the FPH enriched pasta

The FPH enriched pasta was examined for starch digestibility, recording reducing sugar levels at 20 min, 60 min, and 120 min. A graph depicting the relationship between released reducing sugar and time is presented (Fig. 2). The different amount of sugar comprises of readily digestible starch (RDS) and slowly digestible starch (SDS) are given in Fig. 3. Additionally, Fig. 4 compares starch hydrolysis values (control vs. FPH-enriched pasta) as area under the curve (AUC).

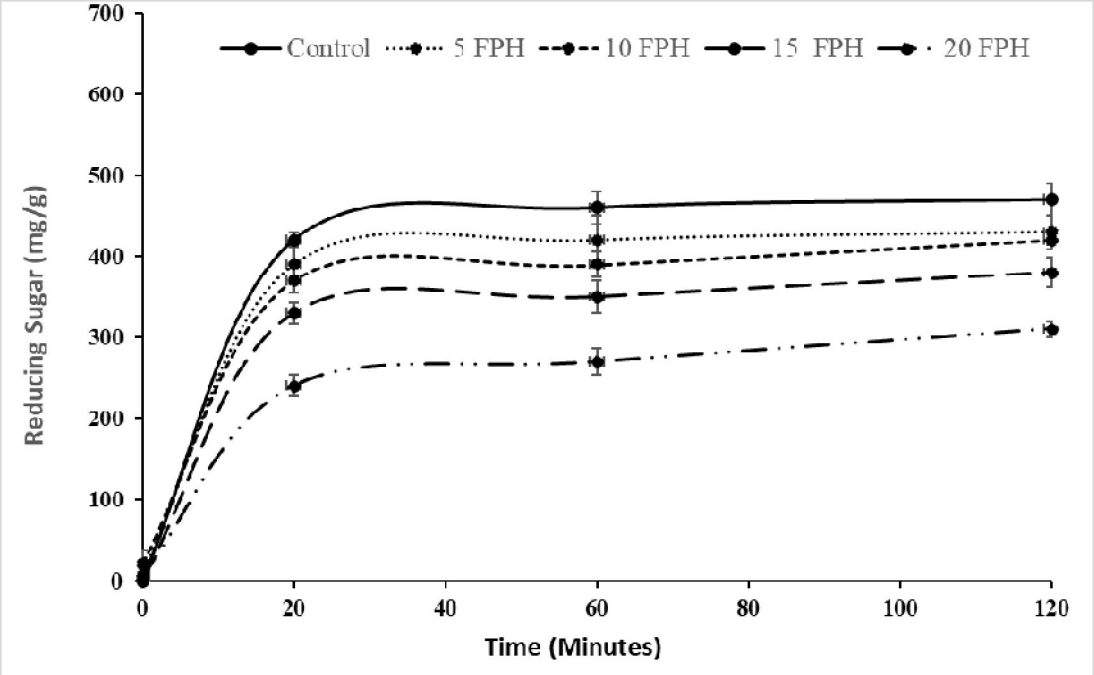

Fig. 2: Amount of reducing sugar released during *in-vitro* digestion of the control and FPH enriched pasta - 5FPH, 10FPH, 15FPH and 20FPH: pasta prepared with 5, 10, 15 and 20 g of fish protein hydrolysate /100 g of semolina flour respectively, CO: control sample.

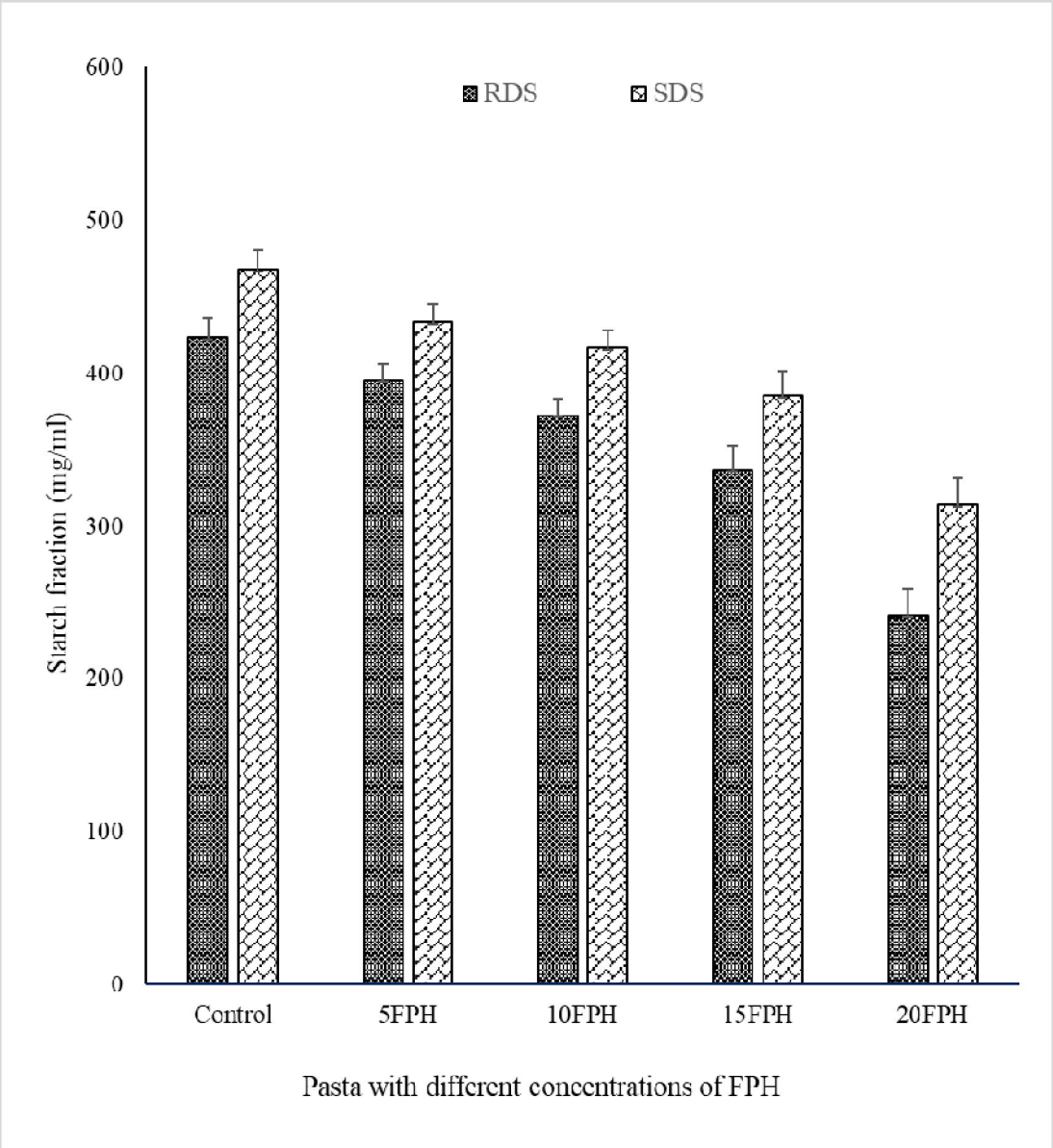

Fig. 3: Readily digestible starch (RDS) and slowly digestible starch (SDS) – starch content hydrolysed within 20 min and 120 min respectively of control and FPH enriched pasta - 5FPH, 10FPH, 15FPH and 20FPH: pasta prepared with 5, 10, 15 and 20 g of fish protein hydrolysate /100 g of semolina flour respectively. The values are expressed as mean ± SD (n=3). The values are significantly different from each other ($P < 0.05$), according to Tukey's test.

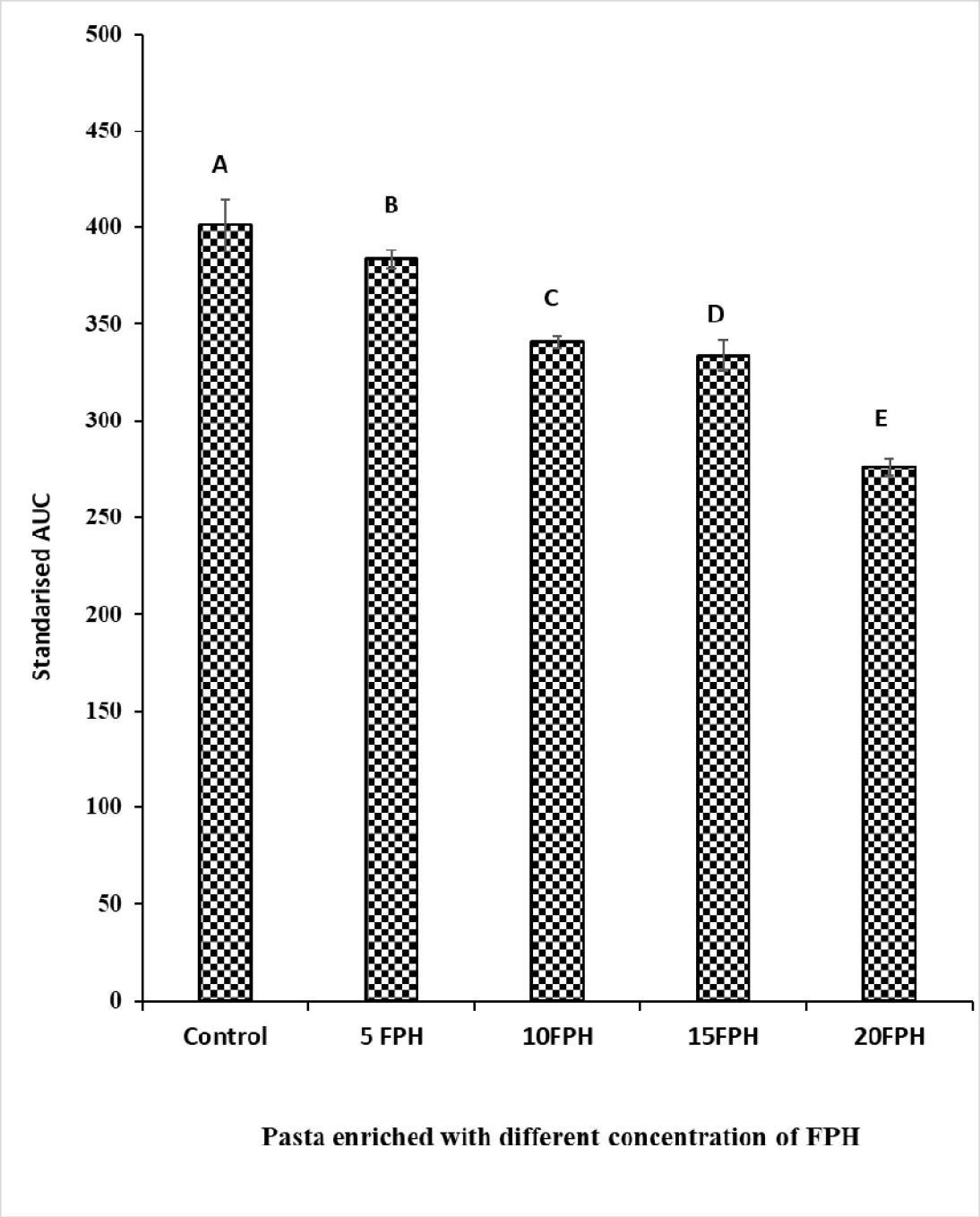

Fig. 4: Values for area under the curve (AUC) comparing control and FPH enriched pasta- 5FPH, 10FPH, 15FPH and 20FPH: pasta prepared with 5, 10, 15 and 20 g of fish protein hydrolysate /100 g of semolina flour respectively. The values are expressed as mean ± SD (n=3). The values are significantly different from each other ($P < 0.05$), according to Tukey's test.

Pasta starch digestibility is crucial for human nutrition, primarily determined by interactions among protein, lipid, and starch in the small intestine, influencing

glycaemicresponse (Ren et al., 2016). The study evaluates the *in-vitro* starch digestibility of FPH enriched pasta by calculating the amount of reducing sugar released over 120 min *in-vitro* digestion. The amount of sugar released in 120 min by the FPH enriched pasta is lower ($P < 0.05$) than the control pasta (Fig. 2). The enriched pasta samples had significantly ($P < 0.05$) lower levels of readily digestible starch (RDS) than slowly digestible starch. It may be due to the complexes formed by lipids with amylose and forms a barrier on the enzyme absorption sites of starch granules by protein (Chen et al., 2017). Similarly, enrichment of semolina spaghetti with protein rich bean flour lowers the RDS and SDS fraction (Giuberti et al., 2015), primarily due to partial gelatinisation of starch granules. The fat content in the pasta sample was 0.48% (control), 0.51% (5% FPH), 0.53% (10% FPH), 0.56% (15% FPH) and 0.58% (20% FPH) respectively. The decrease in starch digestibility may be due to the formation of amylose-lipid complexes (ALC) which is supported by the statement given by Ren et al. (2016) that ALC is resistant to enzyme and the resistant capacity increases with increasing lipid chain length. Also, hydration and swelling of starch granules interrupted by lipid-amylose interaction, forming a single helical structure that obstructs the enzymatic hydrolysis of the starch granules. Lau et al. (2016) found the addition of fats in baked bread and performed starch digestibility test where a significant reduction in the digestibility was found with formation of amylose-lipid complex. The incorporation of stearic acid and linoleic acid in bambara starch reduced starch digestibility with the formation of single helical structure of amylose-lipid complex (Oyeyinka et al., 2017). Similarly, the *in-vitro* starch digestibility and glycaemic index of millets comprises of oleic, palmitic and linoleic acids (Annor et al., 2015). Moreover, in high protein sample reduction of starch granule surface accessibility area occurs that affects the enzyme susceptibility. The present study was carried out with protein rich FPH enriched pasta. Therefore, there could be formation of strong protein network that can entrap α-amylase (Singh et al., 2010). Protein and lipid content in cereal products reduce starch digestibility by forming starch-lipid and starch-lipid-protein complexes (Kadam and Prabhasankar, 2012).A study investigated the effect of freeze-dried shrimp meat on pasta starch digestibility, revealing a significant decrease compared to durum wheat semolina pasta. Pasta containing freeze-dried shrimp meat (2.5%, 5%, and 10%) showed digestibilities of 60%, 50%, and 38%, respectively, compared to 72% for control pasta. The reduced digestibility is attributed to shrimp protein entrapping starch granules in the gluten network and inhibiting α-amylase activity (Ramya et al., 2015). The protein creates a strong network in the food matrix thereby reducing the surface area of the starch granule for enzyme activity (Rosa-Sibakov et al., 2016).

The effect of substituting semolina flour with FPH on standardised area under the curve (AUC) values with the control (100% durum wheat semolina) sample was illustrated in

the result (Fig. 4). The AUC values in the graph were significantly declined ($p<0.05$) with increasing percentage of FPH in the pasta. A similar pattern was also found in the development of spaghetti by replacing semolina with soya bean flour which shows the impact of decreasing carbohydrate intake on lowering the glycaemic response (Chillo et al., 2010). Hence, control pasta was more digestible than FPH enriched pasta. For lowering the glycaemic index, addition of FPH in carbohydrate rich product like pasta is a wise choice.

3.3 Amino acid composition of FPH treated pasta

The amino acid profiling was carried out for different level of FPH treated pasta sample. The amino acid present are listed in Table 2.

Table 2: Amino acid content (mg/g) of control pasta and pasta enriched with different concentration of FPH

Amino acid	CO	5FPH	10FPH	15FPH	20FPH
Essential amino acid					
Methionine	2.17 ± 0.07 ^{a}	2.65 ± 0.1 ^{b}	3.46 ± 0.4 ^{c}	3.45 ± 0.2 ^{c}	4.08 ± 0.2 ^{d}
Isoleucine	1.84 ± 0.01 ^{a}	3.79 ± 0.5 ^{b}	3.66 ± 0.2 ^{c}	3.68 ± 0.07 ^{c}	4.15 ± 0.1 ^{d}
Leucine	1.27 ± 0.05 ^{a}	1.69 ± 0.2 ^{b}	1.66 ± 0.2 ^{b}	1.9 ± 0.1 ^{c}	5.55 ± 0.1 ^{d}
Lysine	7.65 ± 0.19 ^{b}	7.43 ± 0.4 ^{a}	7.55 ± 0.2 ^{b}	8.53 ± 0.1 ^{c}	9.02 ± 0.2 ^{d}
Threonine	1.76 ± 0.09 ^{b}	1.63 ± 0.2 ^{a}	1.68 ± 0.1 ^{a}	2.52 ± 0.2 ^{c}	2.58 ± 0.09 ^{c}
Tryptophan	1.03 ± 0.15 ^{a}	1.65 ± 0.2 ^{c}	1.51 ± 0.2 ^{b}	2.12 ± 0.03 ^{d}	2.38 ± 0.1 ^{d}
Histidine	0.99 ± 0.02 ^{a}	1.57 ± 0.2 ^{b}	2.22 ± 0.1 ^{c}	2.48 ± 0.1 ^{d}	2.64 ± 0.07 ^{e}
Valine	0.37 ± 0.04 ^{a}	1.56 ± 0.2 ^{c}	1.44 ± 0.07 ^{b}	2.05 ± 0.5 ^{d}	2.15 ± 0.1 ^{e}
Total	17.08 ± 1.34 ^{a}	21.97 ± 2.34 ^{b}	23.18 ±1.83 ^{c}	26.73 ±1.74 ^{d}	32.55 ± 1.14 ^{e}
Non-essential amino acids					
Aspartic acid	4.81 ± 0.13 ^{a}	9.43 ± 0.2 ^{b}	10.4 ± 0.2 ^{c}	16.8 ± 0.4 ^{d}	25.7 ± 0.3 ^{e}
Glutamic acid	22.21 ± 0.28 ^{a}	29.11 ± 0.4 ^{b}	33.75 ± 0.1 ^{c}	47.2 ± 0.8 ^{d}	59.41 ± 1.04 ^{e}
Cysteine	1.38 ± 0.18 ^{a}	2.46 ± 0.3 ^{b}	3.7 ± 0.1 ^{c}	6.47 ± 0.3 ^{d}	8.84 ± 0.1 ^{e}
Serine	0.27 ± 0.06 ^{a}	0.32 ± 0.09 ^{b}	0.63 ± 0.1 ^{c}	4.65 ± 0.1 ^{d}	9.59 ± 0.2 ^{e}
Glutamine	0.80 ± 0.08 ^{a}	0.87 ± 0.07 ^{a}	1.19 ± 0.09 ^{b}	2.37 ± 0.1 ^{c}	4.7 ± 0.3 ^{d}
Glycine	19.11 ± 0.14 ^{a}	21.29 ± 0.4 ^{b}	24.58 ± 0.1 ^{c}	35.29 ± 0.8 ^{d}	65.34 ± 0.5 ^{e}
Arginine	0.85 ± 0.02 ^{a}	1.48 ± 0.2 ^{b}	6.6 ± 0.06 ^{c}	6.83 ± 0.2 ^{d}	13.86 ± 0.6 ^{e}
Alanine	6.23 ± 0.19 ^{a}	7.41 ± 0.1 ^{b}	7.81 ± 0.1 ^{b}	8.88 ± 0.1 ^{c}	17.54 ± 0.3 ^{d}
Tyrosine	6.16 ± 0.29 ^{a}	8.78 ± 0.2 ^{b}	10.71 ± 0.04 ^{c}	12.76 ± 0.3 ^{d}	25.79 ± 0.3 ^{e}
Total	61.82 ± 3.12 ^{a}	81.15 ± 2.67 ^{b}	99.37 ± 3.96 ^{c}	141.25 ± 4.12 ^{d}	230.77 ± 2.45 ^{e}

5FPH, 10FPH, 15FPH and 20FPH: pasta prepared with 5, 10, 15 and 20 g of fish proteinhydrolysate /100 g of semolina flour respectively. CO: control sample. Mean ± SD^{a,b,c,d,e} (n = 3) within a raw followed by the same superscript letter are not significantly different from each other ($P < 0.05$), according to Tukey's test.

Addition of fish protein hydrolysate to pasta product increased ($P < 0.05$) the levels of essential amino acids (EAA) such as methionine, lysine, tryptophan, threonine, isoleucine, leucine, valine, histidine, and some non-essential amino acids (NEAA) including aspartic

acid, glutamic, cysteine, serine, glutamine, glycine, arginine, alanine, and tyrosine. In the EAA, methionine, isoleucine, leucine, and lysine were increased ($P < 0.05$) in the pasta incorporated with FPH (5 to 20 g/kg) as compared to control pasta. While, content of tryptophane, histidine and valine were higher ($P < 0.05$) in the fortification containing 45%, 37% and 17% of control pasta respectively. No difference ($P > 0.05$) was observed in the threonine contents. Amongst the NEAA, aspartic acid, glutamic acid, glycine, arginine, alanine and tyrosine levels were increased ($P < 0.05$) in a level- dependent manner of FPH addition to pasta. While, cystine, serine and glutamic content was found higher ($P < 0.05$) in 5FPH, 10FPH, 15FPH and 20FPH than control pasta sample (without FPH). With the enrichment of FPH, the content of essential and non-essential amino acid in the pasta were found improving with the increase in the amount of FPH added. In agreement with our results, Monteiro et al. (2016) and Seda (2014) reported similar pattern for total EEA and NEAA levels in tilapia (Oreochromis niloticus) flour fortified pasta and Tincatinca mince incorporated bread, respectively. However, the results indicated a decrease in tryptophan, histidine, and methionine contents due to the fortification of tilapia flour and T. tinca mince, contrary to current findings. Desai et al. (2018b) reported a higher level of amino acids in pasta made from cod fish powder compared to semolina-made pasta. Similarly, the powder of whole herring, herring by-products and arrowtooth flounder fillet has also shown increasing the pattern of amino acid (Sathivel et al., 2004).

Fatty acid profile of FPH enriched pasta

The fatty acid profile of pasta enriched with different levels of FPH and control pasta (without FPH) were depicted in Table 3.

Table 3:Fatty acid profile (g of individual fatty acids/100g of total fatty acids) of pasta enriched with different levels of FPH

Fatty acid	Control	5FPH	10 FPH	15FPH	20FPH
Saturated Fatty Acids (SFA)					
C12:0	2.51 ± 0.01^{d}	2.59 ± 0.02^{d}	2.06 ± 0.01^{c}	1.82 ± 0.00^{b}	1.68 ± 0.00^{a}
C14:0	7.62 ± 0.06^{e}	5.61 ± 0.07^{d}	5.14 ± 0.04^{c}	4.56 ± 0.01^{b}	4.16 ± 0.01^{a}
C15:0	1.75 ± 0.01^{e}	1.35 ± 0.01^{d}	1.10 ± 0.01^{c}	0.93 ± 0.01^{b}	0.82 ± 0.01^{a}
C16:0	24.46 ± 1.44^{a}	30.77 ± 0.08^{e}	28.32 ± 0.05^{d}	27.12 ± 0.03^{c}	26.23 ± 0.03^{b}
C17:0	0.85 ± 0.08^{e}	0.74 ± 0.08^{d}	0.61 ± 0.04^{c}	0.55 ± 0.04^{b}	0.44 ± 0.04^{a}
C18:0	7.16 ± 0.11^{c}	7.24 ± 0.10^{d}	6.48 ± 0.01^{c}	6.18 ± 0.01^{b}	6.04 ± 0.01^{a}
C19:0	0.18 ± 0.01^{b}	0.21 ± 0.01^{c}	0.14 ± 0.02^{a}	0.18 ± 0.02^{b}	0.17 ± 0.01^{b}
C20:0	0.12± 0.01^{b}	0.11± 0.03^{a}	0.12 ± 0.02^{b}	0.11 ± 0.02^{a}	0.10 ± 0.01^{a}

C22:0	-	0.06± 0.00 [a]	0.11± 0.01 [b]	0.15 ± 0.01 [c]	0.16 ± 0.01 [c]
C24:0	-	0.07 ± 0.01 [a]	0.09 ± 0.02 [a]	0.11 ± 0.01 [c]	0.13 ± 0.01 [b]
Monounsaturated Fatty Acids (MUFA)					
C14:1	0.80 ± 0.04 [e]	0.61 ± 0.03 [d]	0.48 ± 0.02 [c]	0.42 ± 0.03 [b]	0.38 ± 0.01 [a]
C16:1 ω-7	1.27 ± 0.02 [a]	2.43 ± 0.01 [b]	3.59 ± 0.01 [c]	3.84 ± 0.00 [c]	3.94 ± 0.01 [c]
C17:1	0.13 ± 0.01 [a]	0.16 ± 0.01 [b]	0.17 ± 0.01 [b]	0.16 ± 0.02 [b]	0.17 ± 0.01 [b]
C18:1 ω-9	16.17 ± 0.08 [d]	21.35 ± 0.12 [b]	26.11 ± 0.01 [c]	33.13 ± 0.05 [d]	36.09 ± 0.04 [e]
C20:1	0.03 ± 0.04 [a]	0.35 ± 0.03 [b]	0.78 ± 0.02 [c]	1.01 ± 0.02 [d]	1.21 ± 0.03 [e]
C22:1 ω-9	-	0.11 ± 0.00 [a]	0.14 ± 0.01 [b]	0.16 ± 0.02 [c]	0.17 ± 0.04 [c]
C24:1 ω-9	-	-	0.07 ± 0.01 [a]	0.08 ± 0.02 [a]	0.08 ± 0.02 [a]
Polyunsaturated fatty acids (PUFA)					
C18:2 ω-6	11.07 ± 0.09 [a]	11.57 ± 0.06 [b]	11.77 ± 0.04 [c]	11.71 ± 0.01 [c]	11.76 ± 0.01 [c]
C18:3 ω-3	1.34 ± 0.00 [a]	1.38 ± 0.01 [b]	1.48 ± 0.01 [e]	1.43 ± 0.00 [d]	1.40 ± 0.00 [c]
C20:2 ω-6	-	0.11 ± 0.01 [a]	0.14 ± 0.00 [b]	0.16 ± 0.00 [c]	0.17 ± 0.00 [d]
C20:3 ω-6	-	0.08 ± 0.01 [a]	0.13 ± 0.00 [b]	0.15 ± 0.00 [c]	0.17 ± 0.00 [d]
C20:4 ω-6	-	0.13 ± 0.01 [a]	0.21 ± 0.02 [b]	0.24 ± 0.04 [c]	0.25 ± 0.06 [d]
C20:5 ω-3	-	0.28 ± 0.01 [a]	0.45 ± 0.05 [b]	0.57 ± 0.07 [c]	0.63± 0.03 [d]
C22:5 ω-3	0.08 ± 0.04 [a]	0.17 ± 0.01 [b]	0.24 ± 0.00 [c]	0.27 ± 0.00 [d]	0.30 ± 0.00 [e]
C22:6 ω-3	-	0.76 ± 0.03 [a]	1.29 ± 0.01 [b]	1.53 ± 0.01 [c]	1.59 ± 0.01 [d]
ΣSFA	44.65 ± 0.45 [c]	48.75 ± 0.41 [d]	44.17 ± 0.27 [c]	41.71 ± 0.12 [b]	39.93 ± 0.11 [a]
ΣMUFA	18.40 ± 0.17 [a]	25.01 ± 0.18 [b]	31.34 ± 0.12 [c]	38.72 ± 0.06 [d]	41.96 ± 0.06 [e]
ΣPUFA	12.49 ± 0.14 [c]	14.48 ± 0.13 [c]	15.71 ± 0.08 [c]	16.06 ± 0.04 [c]	16.27 ± 0.04 [c]
Σ ω-6	11.07 ± 0.01 [a]	11.89 ± 0.02 [b]	12.25 ± 0.05 [c]	12.26 ± 0.07 [c]	12.35 ± 0.08 [d]
Σ ω-3	1.42 ± 0.01 [a]	2.59 ± 0.04 [b]	3.43 ± 0.03 [c]	3.80 ± 0.02 [d]	3.92 ± 0.02 [d]
PUFA/SFA	0.27	0.29	0.35	0.38	0.40
ω-6/ ω-3	7.79	4.45	3.57	3.22	3.15

5FPH,10FPH,15FPH and 20FPH: Pasta prepared with 5, 10, 15 and 20 g of FPH /100 g of semolina. Control pasta sample. Mean ± SD (n = 3).Values within a column followed by the same superscript letter are not significantly different from each other ($P < 0.05$), according to Tukey's test.

In this study, elevating the FPH concentration in pasta led to a decline in saturated fatty acid (SFA) levels ($P > 0.05$), while increased level ($P < 0.05$) of mono-unsaturated fatty

acid (MUFA) and polyunsaturated fatty acid (PUFA) was found. This reflects a positive impact of addition of FPH on pasta quality which will ultimately improves health of consumer. FPH fortification in pasta increased the content of palmitoleic acid (C16:1), and vaccenic acid (C18:1) than the control pasta and resulting on increased ($P < 0.05$) of total MUFA content. The major PUFA present in control pasta was linoleic acid (C18:2). The major PUFA present in control pasta was linoleic acid (18:2) and omega-3 fatty acids were low in content with the ratio of ω-6: ω -3 being 7.79. The higher ω-6: ω-3 ratio in control pasta could be due to the prevalence of linoleic acid in pasta made with semolina (Fradique et al., 2013). The pasta prepared with different proportion of FPH showed the ratio of ω-3: ω-6 fatty acids increased ($P < 0.05$) from 4.45 to 3.15. The recommended ratio on ω-6: ω-3 is 1 - 5 as specified by food agencies, scientific societies and national and international organisations (Agostoni et al., 2010) and also added that from human nutrition standpoint and prevention of cardiovascular disease, a food with ratio of ω-6: ω-3 is 1 - 5 is recommended. Researchers also proved that ω-6: ω-3 ratio of cereal based enriched product can also enhance with the inclusion of Japanese seaweed (*Undariapinnatifida*) (El-beltagi et al., 2017), shrimp meat (*Penaeus monodon*) (Prabhasankar et al., 2009) and carp fish powder (*Cyprinus carpio*) (Ramya et al., 2015). The consumption of FPH enriched pasta can also meet the daily requirement of EPA and DHA. The intake of ω-3 LC-PUFA rich food helps in the prevention of colon cancer and improves insulin sensitivity (Shahidi and Ambigaipalan, 2018). Future studies about the effects of drying and cooking processes of the pasta on EPA and DHA content are necessary. Our findings can be attributed to the difference between fatty acid composition of semolina and FPH. Pasta products contain mostly carbohydrates and exhibit low fatty acid content while FPH presents elevated levels of MUFA and PUFA (Monterio et al., 2016).

4. CONCLUSION

The amino acid and fatty acid content of the pasta was found increased with the incorporation of FPH consists of PUFAs with better protein and starch digestibility. Therefore, fish waste can be utilized for the development of food hydrocolloids, fish protein hydrolysate with rich in nutrient source instead of discarding it in the environment that leads to environmental pollution which in turn effects human health. Hence, there is a huge scope and demand for development of cereal-based product (pasta) with protein rich hydrocolloids, fish protein hydrolysate.

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1.

2.

3.

REFERENCES

1. Agostoni, C., Bresson, J. L., Chardigny, J. M., 2010. Outcome of the public consultation on the draft opinion of the scientific panel on dietetic products, nutrition, and allergies (NDA) on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated. EFSA J. 8(5), 1–232.
2. Ainsa, A., Marquina, P.L., Roncalés, P., Beltrán, J.A. & Calanche M., J.B. (2021). Enriched fresh pasta with a sea bass by-product, a novel food: Fatty acid stability and sensory properties throughout shelf life. Foods, 10.

3. Annor, G.A., Marcone, M., Corredig, M., Bertoft, E., Seetharaman, K., 2015. Effects of the amount and type of fatty acids present in millets on their in vitro starch digestibility and expected glycemic index (eGI). J. Cereal Sci. 64, 76-81.
4. Brennan, C.S., Kuri, V. and Tudorica, C.M., 2004. Inulin-enriched pasta: effects on textural properties and starch degradation. *Food Chemistry*, 86(2), pp.189-193.
5. Carbonaro, M., Maselli, P., Nucara, A., 2012. Relationship between digestibility and secondary structure of raw and thermally treated legume proteins: a Fourier transform infrared (FT-IR) spectroscopic study. Amino acids, 43, 911-921.
6. Chen, X., He, X.W., Zhang, B., Fu, X., Jane, J.L., Huang, Q., 2017. Effects of adding corn oil and soy protein to corn starch on the physicochemical and digestive properties of the starch. Int. J. Biol. Macromol. 104,.481-486.
7. Chillo, S., Monro, J.A., Mishra, S., Henry, C.J., 2010. Effect of incorporating legume flour into semolina spaghetti on its cooking quality and glycaemic impact measured in vitro. Int. J. Food Sci. Nutr. 61(2), 149-160.
8. Damodaran, S., 2008. Amino acids, peptides and proteins. Fennema's Food Chem. 4, 217-329.
9. De Marco, E.R., Steffolani, M.E., Martínez, C.S., León, A.E., 2014. Effects of spirulina biomass on the technological and nutritional quality of bread wheat pasta. LWT-Food Sci. Technol. 58(1),102-108.
10. Desai, A., Brennan, M.A., Brennan, C.S., 2018a. The effect of semolina replacement with protein powder from fish (*Pseudophycisbachus*) on the physicochemical characteristics of pasta. LWT-Food Sci. Technol. 89, 52-57.
11. Desai, A.S., Beibeia, T., Lu, X., Gao, J., Brennan, M., Guo, X., Zeng, X.A. and Brennan, C., 2021. How the inclusion of cod (Pseudophycisbachus) protein enriched powder to bread affects the in vitro protein and starch digestibility, amino acid profiling and antioxidant properties of breads. European Food Research and Technology, 247(5), pp.1177-1187.
12. Desai, A.S., Brennan, M.A. & Brennan, C.S. (2019). Influence of semolina replacement with salmon (Oncorhynchus tschawytscha) powder on the physicochemical attributes of fresh pasta. International Journal of Food Science and Technology, 54, 1497–1505.
13. Desai, A.S., Brennan, M.A., Brennan, C.S., 2018b. Effect of fortification with fish (Pseudophycisbachus) powder on nutritional quality of durum wheat pasta. Foods, 7(4), 62-73.

14. Desai, A.S., Brennan, M.A., Guo, X., Zeng, X.A., Brennan, C.S., 2019. Fish protein and lipid interactions on the digestibility and bioavailability of starch and protein from durum wheat pasta. Molecules, 24(5), 839-851.
15. El-Beltagi, H. S., El-Senousi, N. A., Ali, Z. A., Omran, A. A., 2017. The impact of using chickpea flour and dried carp fish powder on pizza quality. PLoS ONE, 12(9), 1–15.
16. Fagundes, G.., Rocha, M. & Salas-Mellado, M.. (2018). Improvement of protein content and effect on technological properties of wheat. Food Research, 2, 221–227.
17. Fradique, M., Batista, A.P., Nunes, M.C., Gouveia, L., Bandarra, N.M., Raymundo, A., 2013. Isochrysisgalbana and Diacronemavlkianum biomass incorporation in pasta products as PUFA's source. LWT-Food Sci. Technol. 50(1), 312-319.
18. Gilani, G.S., Xiao, C.W., Cockell, K.A., 2012. Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. Br. J. Nutr. 108, 315-S332.
19. Giuberti, G., Gallo, A., Cerioli, C., Fortunati, P. and Masoero, F., 2015. Cooking quality and starch digestibility of gluten free pasta using new bean flour. Food Chemistry, 175, pp.43-49.
20. Gopalakrishnan, J., Menon, R., Padmaja, G., Sajeev, M.S. & Moorthy, S.N. (2011). Nutritional and functional characteristics of protein-fortified pasta from sweet potato. Food and Nutrition Sciences, 2, 944–955.
21. Greco, E., Winquist, A., Lee, T.J., Collins, S., Lebovic, Z., Zerbe-Kessinger, T., Jahan Mihan, A., 2017. The role of source of protein in regulation of food intake, satiety, body weight and body composition. J. Nutri. Health Food Eng. 6(6),186-193.
22. Hsu, H., Vavak, D., Satterlee, L., Miller G., 1977. A multienzyme technique for estimating protein digestibility. J. Food Sci. 42, 1269-1273.
23. Islam, M.S., Hongxin, W., Admassu, H., Noman, A., Ma, C., An Wei, F., 2021. Degree of hydrolysis, functional and antioxidant properties of protein hydrolysates from Grass Turtle (Chinemysreevesii) as influenced by enzymatic hydrolysis conditions. Food Sci. Nutr. 9(8), 4031-4047.
24. Kadam, S.U., Prabhasankar, P., 2012. Evaluation of cooking, microstructure, texture and sensory quality characteristics of shrimp meat-based pasta. J. Texture Stud. 43(4), 268-274.

25. Kristinsson, H.G. and Rasco, B.A., 2000. Fish protein hydrolysates: production, biochemical, and functional properties. Critical Reviews in Food Science and Nutrition, 40(1), pp.43-81.
26. Labuckas, D.O., Maestri, D.M., Perello, M., Martínez, M.L., Lamarque, A.L., 2008. Phenolics from walnut (Juglans regia L.) kernels: Antioxidant activity and interactions with proteins. Food Chem. 107(2), 607-612.
27. Laleg, K., Barron, C., Cordelle, S., Schlich, P., Walrand, S. &Micard, V. (2017). How the structure, nutritional and sensory attributes of pasta made from legume flour is affected by the proportion of legume protein. LWT - Food Science and Technology, 79, 471–478.
28. Lau, E., Zhou, W., Henry, C.J., 2016. Effect of fat type in baked bread on amylose–lipid complex formation and glycaemic response. Br. J. Nutr. 115(12), 2122-2129.
29. Li, M., Zhu, K.X., Guo, X.N., Brijs, K., Zhou, H.M., 2014. Natural additives in wheat-based pasta and noodle products: opportunities for enhanced nutritional and functional properties. Compr. Rev. Food Sci. Food Saf. 13(4), 347-357.
30. Lorusso, A., Verni, M., Montemurro, M., Coda, R., Gobbetti, M., Rizzello, C.G., 2017. Use of fermented quinoa flour for pasta making and evaluation of the technological and nutritional features. LWT-Food Sci. Technol. 78, 215-221.
31. Monteiro, M.L.G., Marsico, E.T., Soares, M.S., Magalhaes, A.O., Canto, A.C.V., Costa-Lima, B.R., Alvares, T.S., Conte, C.A., 2016. Nutritional profile and chemical stability of pasta fortified with tilapia (Oreochromis niloticus) flour. PloS one, 11(12), 1371-1383.
32. Oyeyinka, S.A., Singh, S., Venter, S.L., Amonsou, E.O., 2017. Effect of lipid types on complexation and some physicochemical properties of bambara groundnut starch. Starch-Stärke, 69(3-4), 1600158.
33. Palmquist, D.L., Jenkins, T.C., 2003. Challenges with fats and fatty acid methods. J. Anim. Sci. 81(12), 3250-3254.
34. Pierre, J.P., Abraham, E.R., Richard, Y., Cleal, J. and Middleton, D.A., 2012. Controlling trawler waste discharge to reduce seabird mortality. Fisheries Research, 131, pp.30-38.
35. Prabhasankar, P., Ganesan, P., Bhaskar, N., Hirose, A., Stephen, N., Gowda, L. R., Miyashita, K., 2009. Edible Japanese seaweed, wakame (Undariapinnatifida) as an ingredient in pasta: Chemical, functional and structural evaluation. Food Chem. 115(2), 501–508.

36. Prodpran, T., Benjakul, S., Phatcharat, S., 2012. Effect of phenolic compounds on protein cross-linking and properties of film from fish myofibrillar protein. Int. J. Biol. Macromol. 51(5), 774-782.

37. Rachman, A., Brennan, M.A., Morton, J. & Brennan, C.S. (2020). Effect of egg white protein and soy protein isolate addition on nutritional properties and in-vitro digestibility of gluten-free pasta based on banana flour. Foods, 9.

38. Ramya, N.S., Prabhasankar, P., Gowda, L.R., Modi, V.K., Bhaskar, N., 2015. Influence of freeze-dried shrimp meat in pasta processing qualities of Indian T. durum wheat. J. Aquat. Food Prod. Technol. 24(6), 582-596.

39. Ren, X., Chen, J., Molla, M.M., Wang, C., Diao, X., Shen, Q., 2016. In vitro starch digestibility and in vivo glycemic response of foxtail millet and its products. Food Funct. 7(1), 372-379.

40. Rosa-Sibakov, N., Heiniö, R.L., Cassan, D., Holopainen-Mantila, U., Micard, V., Lantto, R., Sozer, N., 2016. Effect of bioprocessing and fractionation on the structural, textural and sensory properties of gluten-free faba bean pasta. LWT-Food Sci. Technol. 67, 27-36.

41. Sathivel, S., Bechtel, P.J., Babbitt, J., Prinyawiwatkul, W., Negulescu, I.I., Reppond, K.D., 2004. Properties of protein powders from arrowtooth flounder (Atheresthesstomias) and herring (Clupea harengus) byproducts. J. Agric. Food Chem. 52(16), 5040-5046.

42. Seda, O., 2014. Evaluation of amino acid changes and crumb hardness of enriched bread with Tench (Tincatinca L., 1758) flesh in Turkey. J. Food Nutr. Res. 2(12), 985-992.

43. Shahidi, F., Ambigaipalan, P., 2018. Omega-3 polyunsaturated fatty acids and their health benefits. Annu. Rev. Food Sci.Technol. 9(1), 1603–1637.

44. Singh, A., Gupta, A., Surasani, V.K.R. & Sharma, S. (2021). Influence of supplementation with pangas protein isolates on textural attributes and sensory acceptability of semolina pasta. Journal of Food Measurement and Characterization, 15, 1317–1326.

45. Singh, J., Dartois, A. and Kaur, L., 2010. Starch digestibility in food matrix: a review. Trends in Food Science and Technology, 21(4), pp.168-180.

46. Singh, J., Dartois, A., Kaur, L., 2010. Starch digestibility in food matrix: a review. Trends Food Sci. Technol. 21(4), 168-180.

47. Snedecor, G. W. and Cochran, W. G. (1967). Statistical methods. Iowa State University Press, Ames, IO, U.S.A.

48. Tejpal, C.S., Vijayagopal, P., Elavarasan, K., Prabu, D.L., Lekshmi, R.G.K., Anandan, R., Sanal, E., Asha, K.K., Chatterjee, N.S., Mathew, S., Ravishankar, C.N., 2021. Evaluation of pepsin derived tilapia fish waste protein hydrolysate as a feed ingredient for silver pompano (Trachinotusblochii) fingerlings: Influence on growth, metabolism, immune and disease resistance. Anim. Feed Sci. Technol. 272, 114748.

49. Wergedahl, H., Liaset, B., Gudbrandsen, O.A., Lied, E., Espe, M., Muna, Z., Mørk, S., Berge, R.K., 2004. Fish protein hydrolysate reduces plasma total cholesterol, increases the proportion of HDL cholesterol, and lowers acyl-CoA: cholesterol acyltransferase activity in liver of Zucker rats. J. nutr. 134(6), 1320-1327.