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AMINO ACIDS PROFILE OF *CLUPEA HARANGUS* FILLET AND CUTOFFS SUBJECTED TO VARIOUS PROCESSING METHODS

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Abstract:

Clupea harangus *(CH) is a table fish locally called sawa in south-western Nigeria. The effect of local processing methods: poaching, wood and charcoal smoking on the amino acid profile of CH fillet, skin, head and bones (SHB) was investigated using standard bioassay methods. The raw fish (fillet and SHB) recorded high glutamic acid concentration ranging from 36.02 to 60.08mg/g and aspartic acid between the range of 20.77 and 36.10mg/g as the most abundant non-essential amino acids (NEAA). Leucine (21.20-34.57mg/g), lysine (16.79-31.57mg/g), alanine (16.82-26.99mg/g) and arginine (24.65-21.53mg/g) were found as the most essential amino acid (EAA) coupled with high proportion of branched-chain amino acids. All the processing methods significantly improved the concentration of EAA in the fish samples ranging from 154.45 to 186.69mg/g (fillet) and 92.32 to 112.97mg/g (SHB) as compared with raw fish samples. Both wood smoking and poaching methods had desirable effects on the amino acid contents in the fillet and SHB. Poached SHB had a high protein quality and a significant source of lysine and sulphur containing EAA that can be explored for human and livestock consumptions. The present study indicated that wood processing or poaching could improve protein quality and amino acids profile of Clupea harangues.*

Keywords: Fish processing; Fish waste; *Clupea harangues*; Amino acids profile

Introduction

Fish contains well-balanced amino acid compositions that consist of ten essential amino acids and ten non-essential amino acids [1]. Amino acids are the fundamental building blocks of protein. They play both metabolic and physiological roles in living organisms, some of which include- formation of muscles, hormones, neurotransmitters and other body tissues. They are also involved in diverse metabolic pathways that have a strong effect on exercise metabolism. Recent studies showed the complex roles of amino acids in the regulation of body composition, bone health, gastrointestinal function, and cell signaling as well as glucose homeostasis [2]. These amino acids are abundant in fish that can be harnessed for human and livestock consumption to meet daily Recommended Dietary Allowance level. Fish is commonly utilized as fish meal, fish sauce and as an animal feed due to its richness in amino acids and thus allowing it to be overpriced [3.] The preparation of fish before processing involves slime removal, beheading, washing, and scaling, gutting, cutting of fins and sometimes removal of bones. Since all parts of fish are enriched with protein, fish waste, therefore, could be beneficial and utilized [4]. It has been well accepted that significant amount of waste (20-80%) such as skin, head and bones were removed during fish preparation mostly depends on the processing method and the fish involved. However, the so-called "waste" could be of substantial values in the production of certain minerals, enzymes, bioactive peptides, collagen and gelatin that could be explored as fish silage, fish meal and fish sauce for both human and livestock.

It is well established that processing of foods thus affects essential amino acids composition and protein nutritional quality though varies depending on the method used [4]. Such effects may perhaps depend on denaturation of the proteins that reduced amino acid availability as a result of cross-linking, racemization, degradation and the formation of complexes with sugars leading to losing of digestibility. Subsequently, fish is usually not consumed raw; the effects of various processing methods employed in preparing them for consumption are of utmost importance [5]. The processing methods have been indicated to deleteriously influencing the protein and amino acid contents of fishes. Considering that fishes are consumed mainly for their protein content and amino acids composition, there is, therefore, the need to look at how these processing methods affect the protein and amino acid content. Studies conducted by Oluwaniyi and Dosunmu [6] reported the effect of local processing methods such as boiling, frying and roasting on the amino acids composition of some fishes consumed in Nigeria. However, there is a lack of scientific information on the effect of poaching or charcoal and wood smoking on the amino acids composition of *Clupea harangues.* The present study, therefore, investigated the impact of poaching, wood and charcoal smoking used for fish processing in Nigeria on the amino acid composition of *Clupea harangues* fillet, skin, head and bones (SHB).

Materials and Methods

Samples processing

The mean length and weight of Clupea harangues were; 30.52 ± 0.22 cm and 197.66 ± 3.67 g, respectively. Freshly harvested fish from the wild sea were purchased from two major fish distributors in Oja-Ipata, Ilorin, packed in ice polystyrene boxes were transported to the laboratory within 30 min. The fish was thoroughly washed and drained, placed on wire gauze and cooked by poaching or smoking (firewood or charcoal). Poaching of the fish was done according to the method described by The Economic Research Service of the USDA [7] modified by Larsen [8]. The procedure followed without the addition of any ingredient. Clupea harangue weighing 7 kg was hot smoked using either firewood or charcoal in Altona smoke kiln as described by FAO/UN [9]. The smoking time, temperature and ambient conditions monitored during the smoking operation. Smoking was terminated when fish was properly dried to an average moisture content of 10.41 ± 0.02%, after 8 h. The fish was turned at intervals and the smoked or poached fish samples kept in cane is woven baskets, under laboratory conditions with no preservative, left to cool and subsequently packaged in low-density and high-density polyethylene bags, respectively, sealed then stored at 8^0C until required for further use. The fish head was removed from the trout and filleting was done manually by cutting the first fillet along the top of the backbone, then flipping the trout over and the second fillet was cut. Fish bones were removed as well as the skin. Fish skin, head and bone (SHB) were collected and ground together.

Fish Sample Hydrolysis

Fish samples were hydrolyzed as described by AOAC [10]. Hydrolysis tubes with samples placed in glass beakers and then hydrolyzed in the oven at 110^0C for 24 h. The tubes were removed and allowed to cool down to room temperature. The content was poured into 2 mL Eppendorf tube and later analyzed for amino acids using LC-MS.

Amino acids analysis

About 0.1 g of the fish samples was defatted using chloroform/methanol and then hydrolyzed using heat at 110 °C in an evacuated sealed ampoule for 24 h. Samples collected were treated with 6M Hcl and 15% phenol [11]. The hydrolysate was allowed to cool down to room temperature, and 2 ml submitted for amino acids analysis using Eppendorf Biotronic, LC 3000 amino acid analyzer (Eppendorf-Biotronic, Hamburg, Germany). The Dumas dry combustion method was used to evaluate amino acids concentration in processed fish diets [12].

Statistical analysis

Significant differences between means of experiments were determined by least significant difference. SPSS 14.0 statistical tool was used to analyze the data obtained [13]. Results were considered statistically significant at a minimum of $P < 0.05$ with Duncan's multiple range tests [14].

Results and discussion

Figure 1: Image Showing Raw and Roasted Samples of *Clupea harengus*

The fish protein contains a well-balanced amino acid composition to balance human daily Recommended Dietary Allowance level. The present study showed that the predominant non-essential amino acids in the raw (NEAA) and processed fish fillet and SHB were glutamic acids and aspartic acid, while lysine, leucine & alanine were the major essential amino acids (EAAs). Some EAAs were less than those present in the standard protein varied from 0.38 to 43.97 mg/g in the fillet as well as 7.96 to 38.48 mg/g protein in the SHB but comparable with the values reported in Atlantic fishes [15, 16] as shown in Tables 1 and 2. The level of total essential, semi-essential and non-essential amino acids for both the raw and processed fish (fillet and SHB) as compared to the Pre-School (2-5yrs) FAO/WHO [17] standard. Processing methods increased the levels of amino acids composition both in the fillet and SHB. The wood smoking method had the highest degree of increase in the fillet but less than poached processing method in the SHB. This result agrees with the reports of El and Kavas [18], and Adeyemi et al. [19] on the influence of processing methods on protein quality and amino acids composition of certain marine fishes.

All the processing methods significantly increased the level of total amino acids (AAs) in the fillet while wood smoked fillet showed the best results. In Nigeria, wood smoking is generally acceptable as a common way of preserving or processing fish for consumption though the mechanism responsible for increasing AAs composition in the fish samples remains unclear. Our findings further support the rationale behind the high acceptance of smoked-dried fish in Nigeria but then need clarity on possible toxicity effect. The three most abundant EAAs in the wood smoked fillets are lysine (10 mg/g), leucine (5.4 mg/g) and threonine (4.4 mg/g) comparable to the raw fillet but slightly lower to the FAO/WHO recommended values [17]. Deficiency of essential amino acids such as lysine, leucine and isoleucine have been reported to cause anemia, retarded growth, lack of energy and hypoglycemia especially in man [20]. Leucine is high in fish protein as reported by Teeri et al. [21] but relatively small in Indian Shark, Silver belly and Sole. An inadequate supply of lysine in the diet may lead to mental and physical handicaps because it is a necessary precursor for the de novo synthesis of glutamate, the most significant neurotransmitter in the mammalian central nervous system [19]. Based on our findings, it is very obvious that proper balance consumption of processed fillets could deter the effect of deficiency [22]. Table 2 shows the effect of processing methods on the EAAs composition in the SHB. The PSHB significantly increased the level of total EAAs as compared with RSHB but markedly lower in relation to standard FAO/WHO recommended dietary allowance. Meanwhile, the amount of total EAAs recorded in PSHB could serve as dietary supplement for low income earners. The magnitude of increase for essential amino acids in the raw cutoffs compared with the FAO/WHO [17] standard are as follows: phenylalanine (9.58 mg/g vs 63 mg/g) < lysine (16.79 mg/g vs 58 mg/g) < leucine (21.2 mg/g vs 58 mg/g) < threonine (11.16 mg/g vs 34 mg/g) < methionine (7.96 mg/g vs 25 mg/g) < isoleucine (10.35 mg/g vs 28 mg/g) < valine (15.28 mg/g vs 35 mg/g) as presented in Table 2.

Wood smoke treatment resulted in increased levels of all the essential amino acids under study (Table 1). Of the essential amino acids in the fillet, threonine increased by 24.19% was the most marked, followed by methionine 21.75% ($P < 0.05$), phenylalanine 21.11%, isoleucine 18.29% ($P < 0.05$), valine 16.39% ($P < 0.05$), and leucine 15.53% as well as lysine 10.28%. This result agrees with that of Erkan et al. [23] who reported similar increase of increase essential contents in marine fish species. Conversely, the poach heat treatment was best method ($P < 0.01$) and resulted in increased levels of all essential amino acids in the SHB (Table 2). Threonine increased by 36.02% was the most marked, followed by Isoleucine 31.85% ($P < 0.05$), valine 23.56%, leucine 23.21% ($P < 0.05$), lysine 21.26% ($P < 0.05$), and methionine 14.70% as well as phenylalanine 6.47%. This result in not in agreement with study by Písaříková et al. [24] on amaranth fish species, who report significant decrease in all essential amino acids with heat treatment. Similarly, in present study semi-essential amino acids were markedly increased in the wood smoked fillet of which serine (6.7 mg/g) and arginine (5 mg/g) were the most abundant. Serine is known in fatty acids metabolism while arginine is essential for children growth and also retards the growth of tumors cancer, a high concentration of these amino acids in WSF could boost the standard functioning of human body and well-being [21].

The levels of cysteine and methionine content in all the groups are considered small as compared to the RDA values. In this work, the predominant non-essential amino acids in the wood smoked fillet were glutamic acid (11.5 mg/g), aspartic acid (7.67 mg/g) and glycine (4.6 mg/g) while other processing methods had no significant effect. This observation was in agreement with the report of Aremu et al. [25] on the smoking effect in the nutritional values of African catfish. Glutamic acid is an excitatory neurotransmitter needed in central nervous coordination in man. Its usefulness cannot be over-emphasized in the treatment of epilepsy, mental retardation, muscular dystrophy, ulcers and hypoglycemic coma [26]. Likewise, aspartic acid plays a vital role in the metabolism, especially in the production of immunoglobulin and antibodies. Amino acid levels increased with all processing methods possibly because increasing kinetic energy during heat processing, causes molecules to vibrate so rapidly and violently thereby disrupting the stability of the hydrogen bonds [27, 28]. The influence of processing methods (poaching and wood smoking) on these amino acids, therefore, enhances their availability for human consumption.

Our previous work showed that removal of head, viscera and other parts of fish may set back the total nutritive value of fish [29]. Similarly, bones from the cut-offs could be a potential source of raw material for health products, calcium or phosphorous source for human consumption as well as animal feed [30]. In this work, the effect of processing methods on amino acids composition in the SHB is presented in Table 2. The level of essential amino acids found in the SHB was less than that recorded in the normal protein, which varies from 7.96 to 38.48mg/g protein. Poached SHB (PSSHB) exhibited the highest ($P < 0.001$) concentration of AAs followed by coal smoked fillet (CSSF). The total essential amino acid (mg/g) in the SHB presented in decreasing order of magnitude: 112.97 > 94.21 > 83.94 mg/g recorded for PSSHB, CSSHB and WSSHB as compared to 83.94 detected in the RSSHB. The total essential amino acid, semi-essential amino acids and non-essential amino acids in the poached SHB was significantly improved, however, the level is more pronounced in the fillet as compared with the values recommended by FAO/WHO [17]. The present work showed that total essential amino acids in the fillet were highest in WSSF (186.69 mg/g) and PSSHB (112.97 mg/g) while lysine was markedly increased (41.12 mg/g) in WSSF as compared to RSSF (31.87 mg/g). Among the non-essential amino acids, glutamate had the highest concentration in the WSSF and CSSHB, having the values of 71.84 mg/g and 39.68 mg/g, respectively. This observation concurred with the findings of Fengzhao et al. [30] and Oluwaniyi et al. [31] who reported a high glutamic acid concentration in the muscles of *Pampus* spp and four species of fish consumed in Nigeria, respectively.

Table 1: Amino acids composition of raw and processed *Clupea harangues* fillet

GROUP (mg/g Dry weight)	RSHB	CSSHB	WSSHB	PSHB	Pre-School (2-5yrs) FAO/WHO (1991)
Lysine	16.79 [b]	19.33 [a]	15.58 [b]	20.36 [a]	58
Methionine	7.96 [b]	9.13 [a]	8.10 [b]	8.82 [a]	25
Threonine	11.16 [c]	12.68 [b]	10.76 [c]	15.18 [a]	34
Isoleucine	10.35 [b]	9.84 [b]	8.56 [b]	13.66 [a]	28
Leucine	21.20 [b]	19.92 [b]	17.54 [c]	26.12 [a]	66
Phenylalanine	9.58 [a]	9.83 [a]	10.20 [a]	9.95 [a]	63
Valine	15.28 [b]	13.48 [c]	13.20 [c]	18.88 [a]	35
Total essential amino acids	92.32 [b]	94.21 [b]	83.94 [c]	112.97 [a]	309
Histidine	1.00 [c]	2.89 [a]	1.85 [b]	1.28 [c]	19
Serine	10.26 [c]	13.67 [a]	12.45 [b]	13.92 [a]	-
Arginine	16.82 [c]	22.42 [a]	17.59 [b]	18.43 [b]	-
Cysteine	0.31 [b]	0.64 [a]	0.40 [b]	0.57 [a]	-
Tyrosine	8.29 [a]	8.61 [a]	8.08 [a]	9.82 [a]	-
Alanine	21.53 [a]	21.64 [a]	18.39 [b]	22.74 [a]	-
Total Semi essential amino acids	58.21 [c]	69.87 [a]	58.76 [c]	66.76 [b]	19
Aspartic acid	20.77 [c]	24.35 [b]	20.66 [c]	25.61 [a]	
Glutamic acid	36.02 [b]	39.68 [a]	34.35 [c]	39.15 [a]	-
Glycine	29.06 [c]	38.48 [a]	31.58 [b]	23.45 [d]	0
Proline	17.84 [b]	21.03 [a]	16.71 [b]	17.31 [b]	0
Total nonessential amino acid	103.69 [b]	123.54 [a]	103.30 [c]	105.52 [b]	0
Total amino acids	150.59 [b]	183.05 [a]	151.59 [b]	146.28 [c]	328

*CSSF: charcoal smoked *sawa* fillet; WSSF: wood smoked *sawa* fillet; PSF: poached *sawa* fillet; RSF: Raw *sawa* fillet; N.D: not detected

Table 2: Amino acids composition of raw and processed *Clupea harangues* cut-offs (SHB: skin, head and bone)

GROUP (mg/g Dry weight)	RSHB	CSSHB	WSSHB	PSHB	Pre-School (2-5yrs) FAO/WHO (1991)
Lysine	16.79 [b]	19.33 [a]	15.58 [b]	20.36 [a]	58
Methionine	7.96 [b]	9.13 [a]	8.1 [b]	8.82 [a]	25
Threonine	11.16 [c]	12.68 [b]	10.76 [c]	15.18 [a]	34
Isoleucine	10.35 [b]	9.84 [b]	8.56 [c]	13.66 [a]	28
Leucine	21.20 [b]	19.92 [c]	17.54 [d]	26.12 [a]	66
Phenylalanine	9.58 [a]	9.83 [a]	10.20 [a]	9.95 [a]	63
Valine	15.28 [b]	13.48 [c]	13.20 [c]	18.88 [a]	35
Total essential amino acids	92.32 [b]	94.21 [b]	83.94 [c]	112.97 [a]	309
Histidine	1.00 [c]	2.89 [a]	1.85 [b]	1.28 [c]	19
Serine	10.26 [c]	13.67 [a]	12.45 [b]	13.92 [a]	-
Arginine	16.82 [c]	22.42 [a]	17.59 [b]	18.43 [b]	-
Cysteine	0.3 1 [d]	0.64 [a]	0.40 [c]	0.57 [b]	-
Tyrosine	8.29 [b]	8.61 [b]	8.08 [b]	9.82 [a]	-
Alanine	21.53 [a]	21.64 [a]	18.39 [b]	22.74 [a]	-
Total Semi essential amino acids	58.21 [b]	69.87 [a]	58.76 [b]	66.76 [a]	19
Aspartic acid	20.77 [b]	24.35 [a]	20.66 [b]	25.61 [a]	
Glutamic acid	36.02 [b]	39.68 [a]	34.35 [c]	39.15 [a]	-
Glycine	29.06 [c]	38.48 [a]	31.58 [b]	23.45 [d]	0
Proline	17.84 [b]	21.03 [a]	16.71[b]	17.31[b]	0
Total nonessential amino acid	103.69 [c]	123.54 [a]	103.30 [c]	105.52 [b]	0
Total amino acids	150.59 [b]	183.05 [a]	151.59 [b]	146.28 [c]	328

*CSSHB: charcoal smoked SHB; WSSHB: wood smoked SHB; PSHB: poached SHB; RSHB: Raw SHB, N.D: Not detected; SHB; Skin, head and bone.

Conclusion

We have showed that processed *Clupea harangue* sold in Ilorin market could serve as significant sources of essential amino acids, especially the cutoffs (skin, head and bone) which proved to be more valuable fish products for obtaining the officially recommended appropriate daily intake of essential amino acids for human. Although the composition of essential amino acids was less advantageous than that in the basic egg protein, the levels were significantly improved after processing via poaching and wood smoking in both the fillet and SHB. Hence, SHB though regarded as waste or cut-off could serve as an excellent protein addition for human diet due to the high content of essential amino acids. Furthermore, the Sulphur containing essential amino acids in the fish products, which were found to be in high amounts in the SHB, can supplement the corresponding deficiency in plant proteins. Thus, the proteins in a mixed diet can be utilized optimally for a healthy body constitution. Wide variations in between fish parts (fillet and SHB) and methods of cooking were observed. Wood treatment was the best (P < 0.01) method in the fillet, whereas poached and coal smoked treatment were the best (P <0.01) methods in the SHB. Further studies are ongoing to investigate deleterious effects of these processing methods on the fish samples for healthy human consumption.

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