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The Effect of Different Thermal Treatments on The Chemical Composition and Amino and Fatty Acids Profiles in Fish Artificial Diets

Jalal M. Al-Noor

Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Basrah- Iraq

*Corresponding Author: e-mail: jalal.essa@uobasrah.edu.iq

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Abstract - The study aimed to investigate the effects of different cooking methods (hot water T1, traditional stove T2 or autoclave cooking T3) on the chemical composition, fatty acid content and amino acid profile of fish diets. Variations in chemical composition moisture, ash, fat, and protein with fatty acid and amino acids profiles were also studied. The variations in chemical composition (moisture, ash, fat, and protein) (fatty acid amino acids) content and composition. The results showed variation in nutritional components with different cooking methods and significant differences ($p < 0.05$) between treatments. Diet-extracted oils contained 15 different fatty acids with varying percentages, with the total saturated fatty acids (SFA) being 23.72%, 23.97%, and 25.00% for diets T1, T2 and T3 respectively. The results indicated that the processed diets contained 18 amino acids, including both essential and non-essential amino acids, with a balanced composition and varying percentages among the processed diets. The total essential amino acids increased from 19.93 µg/100 µg protein in diet T3 to 20.36 µg/100 µg protein in diet T1 and 20.23 µg/100 µg protein in diet T2. The study concluded the importance of selecting the appropriate processing method for fish diet manufacturing and highlighted the need to examine changes in the chemical properties of nutritional components and the impact of temperature on nutrient digestibility and fish diet utilization., It seems that diet prepared using only hot water gave the best results.

تأثير المعاملات الحرارية المختلفة على التركيب الكيميائي وصورة الأحماض الأمينية والدهنية لعلائق الأسماك

جلال محمد عيسى النور
وحدة الاستزراع المائي، كلية الزراعة، جامعة البصرة، البصرة – العراق

المستخلص - هدفت الدراسة لتحقق من تأثير طرق حرارة الطبخ المختلفة (الطبخ بالماء الساخن فقط T1 ، الطبخ بالطباخ فقط T2، الطبخ بالأوتوكليف T3) على التركيب الكيميائي ومحتوى الأحماض الدهنية والامينية لعلاق الاسماك، تم متابعة التغيرات في التركيب الكيميائي (الرطوبة والرماد والدهن والبروتين) ومحتوى وتركيب الأحماض الدهنية والأحماض الأمينية، أظهرت النتائج تبايناً في المكونات الغذائية باختلاف طرق طبخ العلائق وبفروق معنوية ($p < 0.05$) بين المعاملات. كما أظهرت النتائج احتواء زيوت العلائق على 15 حامضاً دهنياً متباينة بنسب تواجدها في الزيت المستخلص، بلغت مجموع نسبة الأحماض الدهنية المشبعة SFA 23.72 و 23.97 و 25.00 % لزيوت العلائق T1 و T2 و T3 على التوالي، وقد بينت النتائج إحتواء العلائق المصنعة على 18 حامضاً أمينياً شملت الأحماض الأمينية الأساسية وغير الأساسية وبتركيبة متوازنة وبنسب تواجد متباينة بين العلائق المصنعة، أرتفع مجموع الأحماض الأمينية الأساسية من 19.93 ميكروغرام/100 ميكروغرام بروتين في عليقة T3 الى 20.36 و 20.23 ميكروغرام/ 100 ميكروغرام بروتين لكل من عليقة T1 و T2 على التوالي. وأستنتج من الدراسة ضرورة معرفة طريقة الطبخ المناسبة لتصنيع الأعلاف السمكية والكشف عن التغيرات في الصفات الكيميائية للمكونات الغذائية وتأثير درجة الحرارة على قابلية هضم المغذيات واستفادة الأسماك منها، وكانت العليقة المحضرة باستخدام الماء الساخن فقط أعطت أفضل النتائج.

الكلمات المفتاحية: الأحماض الأمينية ، الأوتوكليف ، الأحماض الدهنية ، علائق اسماك، حرارة

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Introduction

The aquafeed industry is a distinguished, diverse, and rapidly growing sector that plays a leading role in the food industry enabling an increase in the economic production of animal-based products worldwide (Khanum *et al*., 2022). Fish feed production is a crucial component in aquaculture, accounting for approximately 60% of the operational costs of fish farms (FAO, 2022).

One of the key challenges in fish nutrition is producing nutritionally balanced feeds that contain all the necessary nutrients for optimal growth, good health, energy production, tissue building, and product quality enhancement (Silva, 2022). The quantitative requirements for any feed largely depend on its composition to provide the energy needed for various vital functions. For protein in the feed to be used for growth and achieve high nutritional efficiency, the supply of energy and essential nutrients must be in proportions that meet the fish's needs for survival and growth (Kolawole and Mustapha, 2023). In feed manufacturing, the mixture of raw materials used in preparation is subjected to thermal processing using hot water or traditional and non-traditional thermal treatments, cooking and extrusion which can either improve their nutritional value or degrade it depending on the temperature used, moisture content of the mixture, and the duration of thermal exposure (Khanom *et al*., 2017).

Extrusion and the use of extruders are among the most common feed manufacturing methods, as they provide optimal conditions of heat, moisture, and pressure (Lundblad *et al*., 2012). Feeds produced by these methods are characterized by high water stability and reduced levels of many anti-nutritional factors, along with more efficient gelatinization of starches, leading to improved digestibility and better utilization by fish, which is considered as cheaper energy source compared to proteins and fats (Romano and Kumar, 2019).

However, negative effects of thermal processing may include protein denaturation or the formation of complexes with other proteins or materials that are difficult for fish to digest, oxidation of proteins, and interaction with reducing substances, which can reduce the protein's response to digestive enzymes or cause loss of amino acids through oxidation (Chen *et al*., 2021).

A deficiency in essential amino acids can lead to poor utilization of dietary protein, converting it into an energy source rather than for growth, thereby reducing the feed's nutritional value and affecting growth (Mmanda *et al*., 2020). Furthermore, suboptimal thermal treatment can reduce the availability of nutrients and digestibility of the feed, which may impact growth rates (Chen *et al*., 2021). Nopiyanti *et al*., (2023) show that cooking processes significantly affect the product's properties, amino acid and fatty acid content, and chemical composition.

Given the nutritional and economic importance of fish feeds, the current study aimed to evaluate the effect of different thermal treatments on the nutritional value of processed fish diets.

Materials and Methods

Diet preparation

Experimental diets were prepared by grinding the dry raw materials separately and then passing them through a sieve with a 0.4 mm diameter mesh (fish meal 25%, soybean 25%, wheat bran 18 %, yellow corn 14%, barley 14%, Vegetable oil 2%, and vitamins and minerals 2%). Subsequently, approximately 100 ml of water was added to every 250 grams of the mixture, and after thorough mixing, three experimental diets were formed after mixing the ingredients to enhance homogeneity. The first diet was treated with hot water only (T1), with mixing and stirring for 15 minutes. The second diet was cooked using a cooker, with continued mixing and stirring for 15 minutes (T2). The third diet was cooked using an autoclave at 121°C and 15 psi for 15 minutes (T3). After the cooking phase, the diets were pressed using a meat grinder with a 2 mm diameter mesh. The pressed diet was left to air dry for 48 hours at room temperature with continuous stirring to remove moisture. Once drying was confirmed to be complete, the diets were broken into pellets and stored in the refrigerator until further testing.

Estimation of chemical composition

The percentage of moisture was determined using an oven at a temperature of 105°C until a constant weight was achieved. The percentage of ash was calculated after burning the samples in a muffle furnace at a temperature of 525°C for 16 hours or until the ash turned white. Total nitrogen was estimated using the semi-micro-kjeldahl method and multiplying the result by a conversion factor for meat 6.25 to obtain the protein percentage. Lipid content was determined using Soxhlet extraction with organic solvent mixture (chloroform: methanol in a ratio of 1:2 (v:v) following the method described by Egan *et al*. (1988). The carbohydrate percentage was calculated mathematically according to AOAC (2006).

Estimation of Amino Acids

The amino acid content of the three diets prepared samples was determined according to the method described by Vidotti *et al*., (2003) An ion exchange column and post-column ninhydrin derivatization were used for analysis, utilizing the Visible-UV Detector -6 Av uv -Spd Shimadzu in an automatic analysis system. High-performance Liquid Chromatography (HPLC) equipment, under the supervision of the Ministry of Science and Technology in Baghdad, Iraq, was employed for this purpose.

Estimation of total fatty acids

The total fatty acid content in the oils extracted from the three diets samples was analyzed using the method described by Abdulkadir *et al*., (2010). The oils were examined using Gas Chromatography-Mass Spectrometry (GC-MS), a comprehensive spectral analysis technique, at the laboratories of the Chemistry Department, the Ministry of Science and Technology, Baghdad, Iraq.

Statistical analysis

The experiment was designed according to the complete randomized design (CRD). The significant differences between treatment means was determined using the least significant difference (LSD) test. All statistical analyses were conducted using the Statistical Package for Social Sciences (IBM SPSS) version 26.0.

Results and Discussion

Results

Table (1) shows the chemical composition (moisture, ash, fat, and protein) of the diets manufactured by different cooking methods. The highest moisture content was recorded in 7.73% for the diet T1, while the lowest was 5.81% for the diet T3. The moisture content for the diet T2 was 6.88%. Statistical analysis revealed significant differences ($p<0.05$) among the treatments. The ash content of the studied diets was similar, ranging from 7.63% in diet T1, which differed significantly ($p<0.05$) from diet T2 and T3, with ash values of 7.89% and 7.93%, respectively.

Regarding fat content, diet T3 had the highest value at 10.61%, while diet T1 and T2 had values of 9.22% and diet 9.94%, respectively. Statistical results showed significant differences ($p<0.05$) among the treatments. For protein, diet T1 had the highest value at 33.18%, followed by diet T2 at 32.82%, which was significantly different ($p<0.05$) from the protein content of diet T3, which was 32.26%. The carbohydrate values showed that diet T1 had the lowest carbohydrate content at 42.24%, while diet T2 had 42.48%, with a significant difference ($p<0.05$) from diet T3, which had a carbohydrate content of 43.39%.

(Table 1) Proximate composition of fish diets

	Moisture	**Ash**	**Crude lipid**	**Crude protein**	**Carbohydrate**
T1	7.73a±0.364	7.63b±0.115	9.22c±0.264	33.18a±0.038	42.24b±0.272
T2	6.88b±0.451	7.89a±0.093	9.94b±0.186	32.82a±0.066	42.48b±0.443
T3	5.81c±0.331	7.93a±0.472	10.61a±0.028	32.26b±0.282	43.39a±0.209

*****Different letters within one column indicate the presence of significant differences at the level ($P< 0.05$).

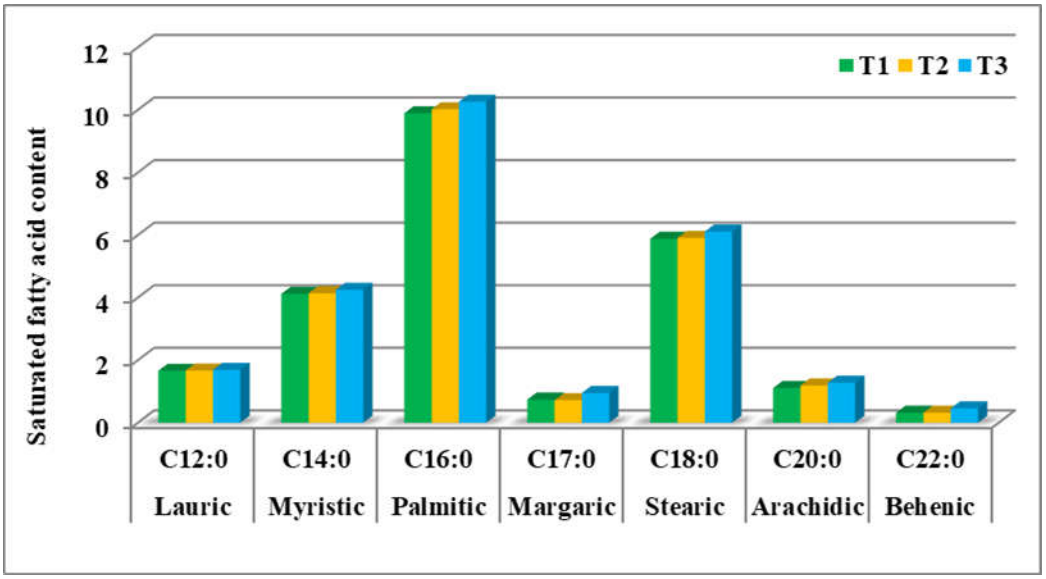
The results in Table (2) and Figures (1) and (2) show the quantity and composition of fatty acids in the diets prepared using different cooking methods through Gas Chromatography-Mass Spectrometry (GC-MS) technique. The results revealed that the diets – extracted oils contained 15 fatty acids with varying concentrations in the extracted oil. The total percentage of saturated fatty acids (SFA) was 23.72%, 23.97%, and 25.00% for diets T1, T2, and T3, respectively.

(Table 2) Fatty acids profiles of fish diets

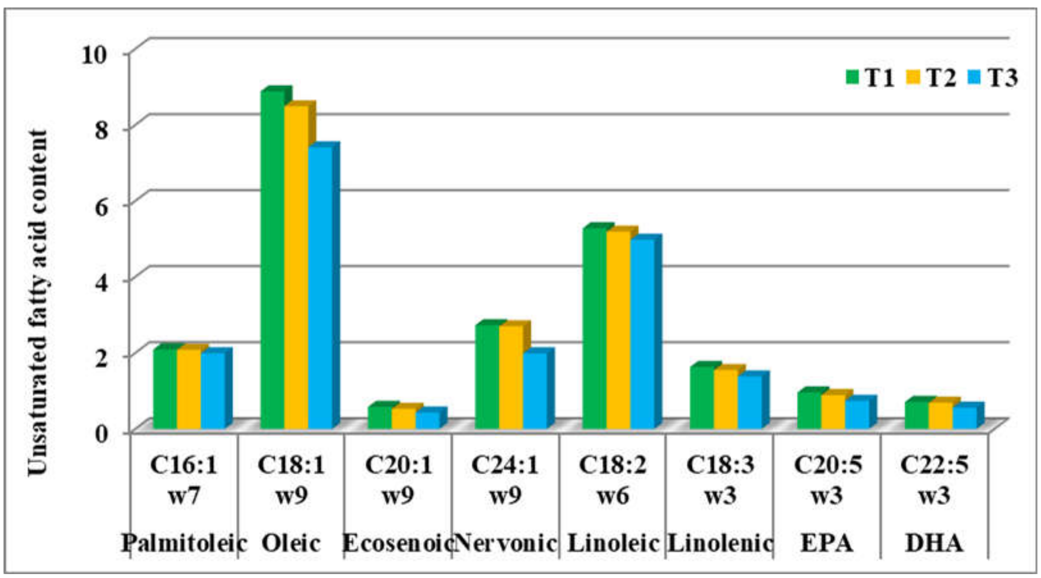
	Fatty acid		**T1**	**T2**	**T3**
Saturated fatty acids SFA	Lauric	C12:0	1.66	1.67	1.69
	Myristic	C14:0	4.12	4.14	4.25
	Palmitic	C16:0	9.89	10.02	10.26
	Margaric	C17:0	0.74	0.72	0.95
	Stearic	C18:0	5.88	5.91	6.11
	Arachidic	C20:0	1.11	1.19	1.28
	Behenic	C22:0	0.32	0.32	0.46
	Σ SFA		**23.72**	**23.97**	**25.00**
Unsaturated fatty acids USFA	Palmitoleic	C16:1 w7	2.09	2.08	1.99
	Oleic	C18:1 w9	8.88	8.49	7.41
	Ecosenoic	C20:1 w9	0.58	0.53	0.43
	Nervonic	C24:1 w9	2.73	2.70	1.99
	Linoleic	C18:2 w6	5.28	5.19	4.98
	Linolenic	C18:3 w3	1.63	1.55	1.38
	EPA	C20:5 w3	0.96	0.89	0.74
	DHA	C22:5 w3	0.71	0.69	0.56
	Σ USFA		**22.86**	**22.12**	**19.48**

Palmitic acid had the highest concentration in the diets, with 9.89%, 10.02%, and 10.26% in diets T1, T2, and T3, respectively, while Behenic acid had the lowest concentration among the different diets, representing 0.32%, 0.32%, and 0.46% for diets T1, T2, and T3, respectively. The levels of monounsaturated fatty acids (MUFA) varied, with the highest total in diet T1 at 22.86%, followed by diet T2 with 22.12%, while diet T3 had the lowest total of MUFAs at 19.48%.

The highest concentration of oleic acid was 8.88%, 8.49%, and 7.41% for diets T1, T2, and T3, respectively, while the lowest concentration of MUFAs was for Ecosenoic acid, with levels of 0.58%, 0.53%, and 0.43% in diets T1, T2, and T3, respectively.



(Figure 1) Saturated fatty acid content of fish diets



(Figure 2) Unsaturated fatty acid content of fish diets

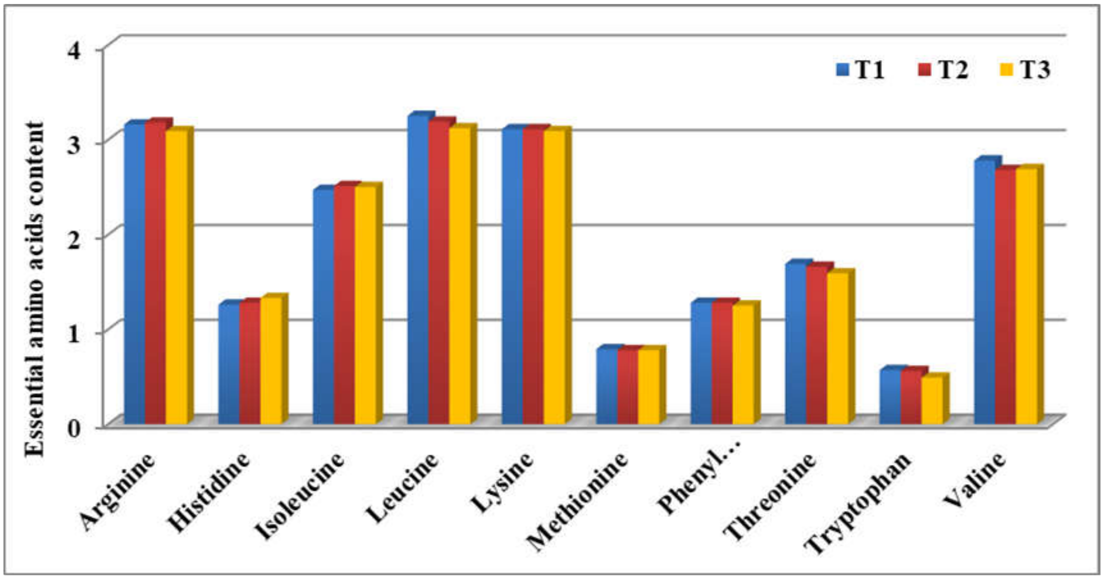
The results shown in Table (3) and Figures (3) and (4) display the amino acid analysis via HPLC for the diet samples processed using different cooking methods. It indicates that the processed diets contained 18 amino acids, including both essential and non-essential amino acids, with a balanced composition and varying concentrations. The total essential amino acids increased from 19.93 µg/100 µg protein in diet T3 to 20.36 and 20.23 µg/100 µg protein for diets T1 and T2, respectively. The amino acid Leucine had the highest values in the processed diets, with concentrations of 3.25, 3.19, and 3.12 µg/100 µg protein for diets T1, T2, and T3, respectively. In contrast, the amino acid Tryptophan was the least abundant across all diets, with concentrations of 0.57, 0.56, and 0.49 µg/100 µg protein for diets T1, T2, and T3, respectively.

Regarding non-essential amino acids, their concentration was highest in diet T1 at 24.11 µg/100 µg protein, and varied in other diets according to the cooking method, with values of 24.03 and 23.92 µg/100 µg protein for diets T2 and T3, respectively.

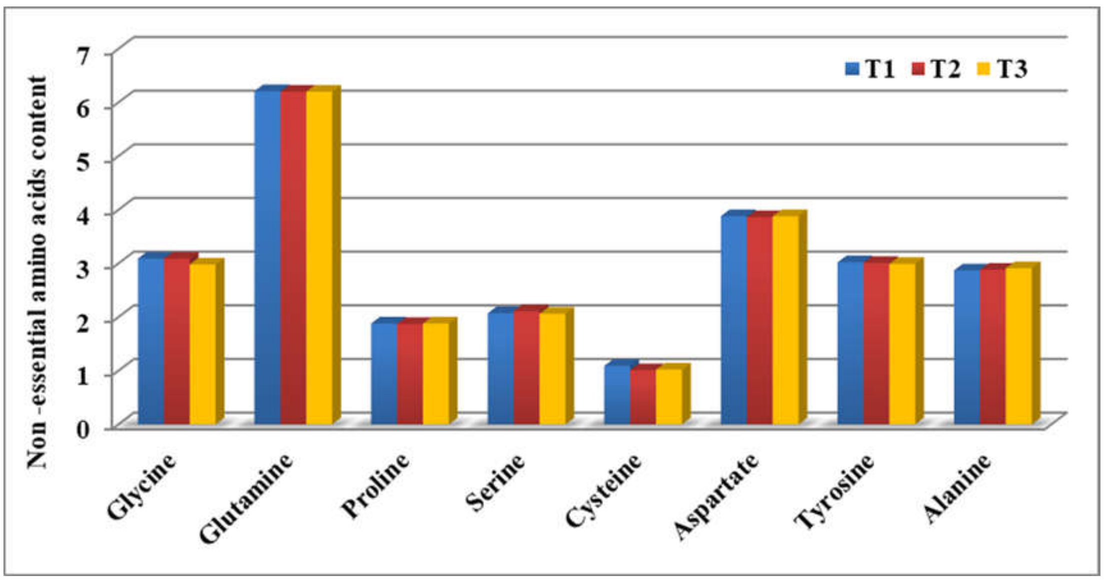
The results also showed that the amino acid Glutamine had the highest concentrations in all processed diets, with values of 6.21, 6.20, and 6.20 µg/100 µg protein for diets T1, T2, and T3, respectively. Conversely, the amino acid Cysteine had the lowest concentration in the processed diets, with values of 1.09, 1.01, and 1.02 µg/100 µg protein for diets T1, T2, and T3, respectively. The remaining amino acids varied in their concentrations across the diets due to differences in processing methods.

(Table 3) Amino acids profiles of fish diets

	Amino acids		**T1**	**T2**	**T3**
Essential amino acids EAA	Arginine	Arg	3.16	3.18	3.09
	Histidine	His	1.26	1.28	1.33
	Isoleucine	Iso	2.47	2.51	2.50
	Leucine	Leu	3.25	3.19	3.12
	Lysine	Lys	3.11	3.11	3.09
	Methionine	Met	0.79	0.78	0.78
	Phenyl alanine	Phe	1.28	1.28	1.25
	Threonine	Thr	1.69	1.66	1.59
	Tryptophan	Trp	0.57	0.56	0.49
	Valine	Val	2.78	2.68	2.69
Σ EAA			**20.36**	**20.23**	**19.93**
Non-essential amino acids NEAA	Glycine	Gly	3.09	3.09	2.98
	Glutamine	Glu	6.21	6.20	6.20
	Proline	Pro	1.88	1.87	1.88
	Serine	Ser	2.07	2.11	2.06
	Cysteine	Cys	1.09	1.01	1.02
	Aspartate	Asp	3.88	3.86	3.88
	Tyrosine	Tyr	3.02	3.01	2.99
	Alanine	Ala	2.87	2.88	2.91
Σ NEAA			**24.11**	**24.03**	**23.92**



(Figure 3) Essential amino acids content of fish diets



(Figure 4) Non-essential amino acids content of fish diets

Discussion

Nopiyanti *et al*., (2023) highlighted the impact of different cooking methods on sensory attributes and chemical composition of foods, leading to new product characteristics. Yu *et al*., (2017) found that different cooking temperatures affect moisture, fat, protein, and ash content, resulting in new chemical composition characteristics. Liu *et al*. (2021) observed fat breakdown and degradation, producing various ketonic and aldehydic compounds that affect the chemical composition. These findings were consistent with Reis (2022), who noted fat loss due to the oxidation of unsaturated fats, which can affect fatty acids depending on the time, temperature, and type of treatment. Liao *et al*. (2017) indicated that at temperatures ranging from 120 to 150°C, protein solubility is lower, thus reducing the chance of protein degradation.

However, high temperatures are undesirable as they can damage proteins, leading to Maillard reaction products such as deoxycephalosporin lysine, which is unavailable to fish. On the other hand, Das *et al*. (2018) reported no significant impact of temperature on protein, organic matter, fat, energy or carbohydrates when using temperatures below 170°C. They found that the optimal extrusion temperature for maintaining diet quality is between 130 and 150°C, which is suitable for starch gelatinization in the diet thus enhancing its physical properties in water and increasing digestibility by fish (Sahu *et al*., 2016). Microwave treatment can alter and break down the molecular properties of difficult-to-digest proteins, increasing their digestibility (Sansuwan *et al*., 2014). Jassim *et al*. (2024) examined the effect of thermal treatment on the chemical composition of common carp diets and observed variability in carbohydrate levels among diets.

They attributed this to the role of heat in breaking down complex carbohydrate molecules, which influences their levels in diets depending on the variation of other nutritional components. Rodríguez-Miranda *et al*. (2014) found that extrusion temperatures affect the chemical composition of fish diets and diet ingredients, resulting in variations in protein, fat, and other nutritional components, Adeleye *et al*. (2020) studied the impact of cooking temperature on the chemical composition and fatty acid profile and observed that crude protein, fat and amino acid contents were negatively affected by temperatures, especially extrusion temperatures, depending on moisture content, temperature, and cooking time. Chu *et al*. (2015) confirmed that thermal treatment of diets can lead to various changes in the chemical and physical properties of diets, including changes in chemical composition, protein denaturation, amylopectin formation, starch gelatinization and Maillard reactions. These changes positively impact carbohydrate digestibility, as effective gelatinization enhances the efficiency of digestive enzymes, improving digestibility and providing a cheaper energy source in diets compared to protein and fat sources (Mohapatra *et al*., 2003).

Nopiyanti *et al*. (2023) reported changes in fatty acid composition, particularly palmitic, oleic, and docosahexaenoic acids, due to different cooking processes, resulting in various new properties of the product. This is consistent with Yu *et al*. (2017) results that fats and fatty acid composition were affected by different cooking temperatures. Abraha *et al*. (2018) noted that the percentage of polyunsaturated fatty acids (PUFA) could be affected by different cooking methods and temperatures. Manzoor *et al*. (2022) explained that extrusion processing could improve the presence and stability of biologically active unsaturated fatty acids and the oxidative stability of fats during storage. Microwave processing is currently used effectively to improve the quality of agricultural by-products for animal diet due to its faster heating rates, shorter processing times, and energy efficiency compared to traditional heating methods such as boiling and steam sterilization (Pokkanta *et al*., 2022). It also inhibits the activity of lipase in raw materials, preventing hydrolysis of triglycerides and the production of glycerol and free fatty acids susceptible to oxidation reactions and undesirable characteristics during storage (Viji *et al*., 2022). Ahmad *et al*. (2022) demonstrated that thermal extrusion and microwave treatments could enhance the quality and content of fatty acids, particularly docosahexaenoic acid (DHA), eicosatetraenoic acid (EPA), and arachidonic acid (AA), as well as improve peroxide value and thiobarbituric acid value when preparing fish diets.

Deng *et al*., (2015) noted that the variation in the concentration and composition of amino acids is attributed to the differences in thermal treatments and thermal exposure time, which can lead to the loss or reduction of certain amino acids due to their sensitivity and decreased moisture content in the food, causing changes in the chemical, functional, and structural properties of the amino acids. In some cases, the levels of amino acids increase with higher temperatures, processing times, and storage due to increased protein degradation, which subsequently raises the concentrations of the resulting amino acids (Rashid *et al*., 2022). However, suboptimal thermal treatment may impair the quality and availability of some nutrients depending on the method used, and some amino acids may become absent after thermal treatment. Proteins can undergo some denaturation or binding under the influence of heat and pressure during extrusion (Chen *et al*., 2021). Rollin *et al*. (2003) confirmed that protein quality depends on the composition and availability of amino acids and their digestibility. Deficiencies in essential amino acids can lead to poor protein utilization, converting it to an energy source rather than supporting growth, which reduces the nutritional value of the diet and lowers growth rates. Nopiyanti *et al*. (2023) noted that different cooking methods and cooking times affect the composition of amino acids, particularly glutamic, aspartic, leucine, and lysine. This finding is consistent with Yu *et al*. (2017), who observed the effects of varying cooking temperatures on proteins and amino acid composition. Abraha *et al*. (2018) mentioned that time, temperature and cooking method are crucial factors that affect proteins, causing denaturation to a degree that could leads to loss of essential amino acids, vitamins and other nutrients.

The results also align with those of Hunsakul *et al*. (2021), who observed increased protein production with higher temperatures, processing times, and storage. However, some amino acids decrease with increased temperature due to their thermal sensitivity. Paes and Maga (2004) found changes in the proportions and contents of amino acids, especially leucine, isoleucine, lysine, threonine, and valine, in some diet materials exposed to manufacturing temperatures. Simultaneously, Ahmad *et al*. (2012) confirmed that suboptimal heat causes proteins to bind with other compounds due to denaturation and oxidation, reducing their digestibility by fish enzymes. Generally, protein digestibility in fish improves when using temperatures lower than 150°C (Morken *et al*., 2012).

Conclusion

In conclusion, it seems that diet prepared using only hot water gave the best results, perhaps due to the moderate temperature of the feed mixture and the short exposure period. This requires attention to the treatments to which the ingredients is exposed during fish diet manufacturing to ensure the best production and economic specifications.

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