ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 12, Issue, 09, pp.13515-13519, September, 2020

DOI: https://doi.org/10.24941/ijcr.39600.09.2020

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

AMINO ACID PROFILE OF TUNA LOIN SPRAYED BY FILTERED SMOKE DURING FROZEN STORAGE

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ARTICLE INFO

Article History:
Received 15th June, 2020
Received in revised form
27th July, 2020
Accepted 04th August, 2020
Published online 30th September, 2020

Key Words:
Filtered Smoke, Tuna loin,
Profile, Amino Acid, Essential.

ABSTRACT

The bright red color of the seafood especially tuna loin is an important quality determination. Filtered smoke was a substance that usually used to maintain the quality of this color. In addition, nutritional quality of tuna also play an important role because of these tuna loin will be consumed and finally will provide benefits for the human body. The purpose of this research is to find out the amino acid profile of tuna loin that has been sprayed by filtered smoke during frozen storage. The research method was an experimental. The first step was treated tuna loin by spraying filtered smoke on it, then stored during 4 weeks in freezing temperatures. The next step was analyzing its amino acid profile at the 1st and the 4th week. The result of the research showed that it has been identified 15 types of amino acids consisted of 9 types of essential amino acids and 6 types of nonessential amino acids. Histidine was the highest essential amino acid in tuna loin treated by filtered smoke, by the number 2.28and 1.6%, for the 1st and 4th weeks stored respectively, while the lowest composition was methionine by the number 0.56% and 0.52% for the 1st and 4th weeks stored respectively. The highest composition of nonessential amino acid of tuna loin treated by filtered smoke was glutamic acid by the number 3.11% and 2.27% for the 1st and 4th weeks stored respectively and the lowest composition of nonessential amino acid was tyrosine by the number 0.8% and 0.11% for the first and fourth weeks stored respectively.

Citation: ***Cindy Regina Magdalena Loppies, Daniel A.N. Apituley, Raja Bonan Dolok Sormin and Beni Setha.*** **2020.** "Amino acid profile of tuna loin sprayed by filtered smoke during frozen storage", *International Journal of Current Research*, 12, (09), 13515-13519.

INTRODUCTION

Tuna is one of fishery commodities of Indonesian which is preferred by foreign consumers because it has a delicious taste and high nutrition. Tuna flesh contains protein 22.6 - 26.2 g/100 g, fat 0.2 - 2.7 g/100 g. In addition, tuna contains the minerals calcium, phosphorus, iron and sodium, vitamin A (retinol), and vitamin B (thiamine, riboflavin and niacin). Biside many advantages, however, tuna also grouped as perishable food, therefore it was needed an effort to prevent the quality of the tuna by good prevention and preserving. The most widely exported tuna from Indonesia is frozen tuna loin (Wicaksono, 2009). Tuna loin is a quarter elongated tuna fillet, consisting of the quarter upper left side fillet, the quarter lower left side fillet, the quarter upper right side fillet and the quarter lower right side fillet, without head, middle bone and fish tail. The advantage of the loin technique is not needed a long time to the manufacturing process, in contrast to the steak technique which takes a long time to process it due to cutting the shape of tuna into small pieces (Junianto, 2003). The most determination factor whether tuna is accepted or not by the consumers is its color. The flesh color of seafood has a very strong influence on consumer acceptance. The bright red color is an important determinant factor of seafood quality, especially tuna. However, after being prepeared and during frozen storage, tuna flesh run into a rapid color changes to be brown color (Anderson and Wu, 2005). In order to maintain the red color of the tuna flesh, the use of carbon monoxide (CO) and Clearsmoke/Filtered smoke (FS) has been applied. (Pivarnik *et al* 2011). Ludlow *et al*, 2004 had reported the research to know the stability of color and bacterial content of tuna flesh (*Thunnus albacares*). However, nowdays, the use of CO in tuna handling does not guarantee the food safety, where the tuna importing countries such as Japan reject the use of this CO. (Faustman and Cassens 1990). The FDA had issued the rule to prohibit the use of CO gas on maintaining the red color in fish flesh (Pivarnik *et al.*, 2011). The replacement of CO to become Filter Smoke/Clear Smoke is associated with the residues in muscle tissue (Kristinssor *et al*, 2006). Handling and storage of fish in freezing temperatures can affect the chemical composition, especially protein.

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Fish protein contains complete essential amino acids, which is needed by the human body. The amino acid content in fish flesh varies greatly, depending on the type of fish. Generally, the amino acid of fish flesh is rich in lysine, but lacks in tryptophan content (Junianto, 2003). Because of the importance of the information about the amino acid profile of tuna loin applied by filter smoke, it is important to study the amino acid profile contained in tuna loin applied by filtered smoke.

MATERIALS AND METHODS

Material

The raw materials used were fresh tuna loin, filtered smoke (FS), chemicals material required in analyzing the tuna loin included: distilled water, NaOH 40%, HCl 0.1N, HCl 0.001N, HCl 10%, H_3BO_3, H_2SO_4, HCl 6 N, kjeltec tablets. Equipments used include knives, buckets, scales, styrofoam, fish grinder, ovens, and a set of laboratory glassware for analysis purposes such as porcelain cups, mortars, ovens, desiccators, kjeltec tubes, erlenmeyers, burettes, soxhlet, micro pipettes and analytical balance.

Research Procedure: Preparing the sample by filleting fresh tuna loin change in to steak shape. Next step put in a plastic container and injected with FS then stored at chilling temperature for 2 days. After chilling, the remained FS is removed from the plastic container and trimmed to vacuum packaging. Before stored, the tuna loin was frozen in air blast freezer (ABF) for 2 days. Finally, tuna loin was stored in cold storage by temperature of -28°C. An observations were carried out 1st week and 4th week. Parameters observed was amino acid profile by using High Performance Liquid Chromatography (HPLC) ICL type with octadecyl silica column (ODS) at the Integrated Laboratory of IPB, Bogor.

The Analysis of Amino Acid: Amino acid analysis begins with hydrolysis of the tuna loin samples that have been applied by FS, using an oven at 110 ºC for 24 hours. The results of the hydrolysis were then added 5 ml of 0.01N HCl and filtered with millipore paper. A number of 10 µl of the sample was derivatized using 25 µl o-phthalaldehyde (OPA) reagent. Sample was prepared by adding 1 ml of 6N HCl into the ampoule containing 3 mg of freeze dried protein. Mixing of 50 mg OPA in 4 ml ethanol and 0.025 ml mercaptoethanol, 0.05 ml Brij30 30%, 1 ml borate buffer 1 mM pH =10.4 for 1 minute.

A number of 5 µl derivatized sample was then injected into the HPLC column, and waited for all the amino acids has been separated, its approximately needed 25 minutes. HPLC operational conditions: Ultra tecsphere column; detector: fluorescence; column temperature: 28ºC and mobile phase flow rate: 1 ml/min. The mobile phase consists of Buffer A (0.025M Na-Acetate; Na-EDTA 0.05%; Methanol 9% and THF 1%) and Buffer B (Methanol 95%). The standard of amino acid used is a mixed standard consisting of 15 types of amino acids, namely: aspartic acid, glutamic acid, serine, histidine, glycine, threonine, arginine, alanine, tyrosine, methionine, valine, phenylalanine, isoleucyne, leucine, and lysine.

RESULT AND DISCUSSION

The Content of Amino Acid of Tuna Loin Applied to Filtered Smoke: Protein is considered to be a macro molecule arranged by a large number of amino acids. Amino acid is one of an organic component containing amino and carboxyl groups. The arrangement of amino acids is unique for every kind of protein (Winarno, 1997), and the arrangement of amino acid in protein indicated the quality of protein. The analysis regarding amino acid profile provided an important information about the composition of essential and non-essential amino acids and also showed the overall amino acid composition which can affect the taste characteristics of the sample analyzed (Pratama, 2011).

The quality of the protein could be assessed from the amino acids arranging the protein. There are two types of amino acids that arrange proteins, namely essential amino acids and non-essential amino acids (Sitompul, 2004). Table 1. show the composition of amino acids of tuna loin that applied by FS during the 1st and 4th weeks of storage. There have been indentified 15 kinds of amino acid in tuna loin treated by FS during the 1st and 4th week of storage on Table 1., they were consisting of 9 essential amino acids namely: histidine, threonine arginine, methionine, valine, phenylalanin, isoleucine, leucine and lysine, and 6 non-essential amino acids namely: aspartic acid, glutamic acid, serine, glycine, alanine, and tyrosine. Essential amino acids are amino acids that cannot be synthesized by the human body, but there are important, so they are obtained from the outside of the human body through food. Amino acids are often called and known as builder substances which are the final result of protein metabolism. Figure 1. stated the essential amino acids of tuna loin. Figure 1. stated that histidine was the highest amino acid obtained from tuna loin by treated FS for the 1st week storage 2.28%, while for the 4th week storage, lysine was consider the highest amino acid obtained 1.68%. Histidine play a role to optimize and maintaining the people health and the growth of infants. Histidine, essential amino acid is obtained from the hydrolysis of proteins found in the sperm of any fish (caviar), this is beneficial for the treating of arthritis and strengthens the connection between nerves especially the nerves of the ear due to hearing.

Histidine is useful for tissue repairing, allergies treating, rheumatoid arthritis, anemia and the formation of red blood cells and white blood cells (Edison 2009). According to FAO/WHO (1985) the requirement of histidin in human body is 0.26% (FAO / WHO 1985 in Santoso *et al.* 1996). Lysine is useful for the human body as antibodies, strengthening the circulatory system of blood, maintaining the growth of normal cells, in accompany with proline and vitamin C to form collagen tissue, decreasing the excess blood triglyceride levels (Harli, 2008 in Liputo *et al*, 2013). According to Rosa and Nunes (2004) arginine, lycine, and leucine are recognize as essential amino acids, they are derive from aquatic animals, therefore they are known as high-protein foods. Lysine is also an essential amino acid that functions as a base. This substance helps in hydrogen bonding and as a general basis in changing the speed of chemical reactions. This amino acid also helps the absorbtion of calcium, muscle protein production, hormone production, recovery from trauma in body tissues and the production of antibodies and enzymes.

Table 1. The composition of amino acid of tuna loin treated by FS

Essential aminoacid	The 1st week	The 4th week
Histidine	2.28 ± 0.02	1.6 ± 0.04
Threonine	1.0 ± 0.10	1.13 ± 0.02
Arginine	1.29 ± 0.01	1.22 ± 0.02
Methionine	0.58 ± 0.02	0.52 ± 0.02
Valine	1.22 ± 0.02	1.08 ± 0.01
Phenylalanine	0.86 ± 0.01	0.76 ± 0.01
Isoleusine	1.11 ± 0.01	0.99 ± 0.01
Leusine	1.72 ± 0.03	1.49 ± 0.01
Lysine	1.91 ± 0.02	1.68 ± 0.02
Total of Essential Amino acid.	11.97	10.47
Non-essential amino acid		
Aspartic acid	2.11 ± 0.02	1.89 ± 0.02
Glutamic acid	3.11 ± 0.01	2.85 ± 0.02
Serine	0.85 ± 0.03	0.70 ± 0.06
Glysine	0.15 ± 0.01	0.86 ± 0.01
Alanine	0.26 ± 0.01	1.08 ± 0.01
Tyrosine	0.8 ± 0.06	0.58 ± 0.01
Total of Non essential amino acid	9.28	8.04
Total of essential and non-essential amino acid.	21.25	18.51

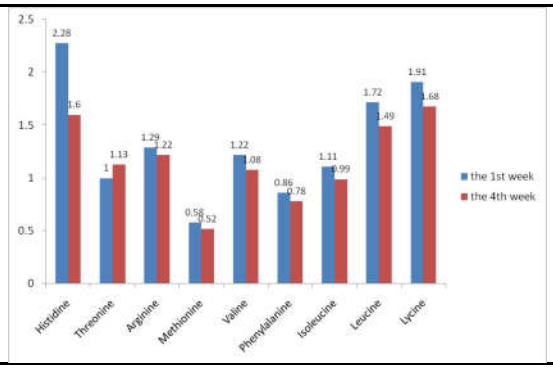

Figure1. Profile of essential amino acids of tuna loin by treated filter smoke during storage

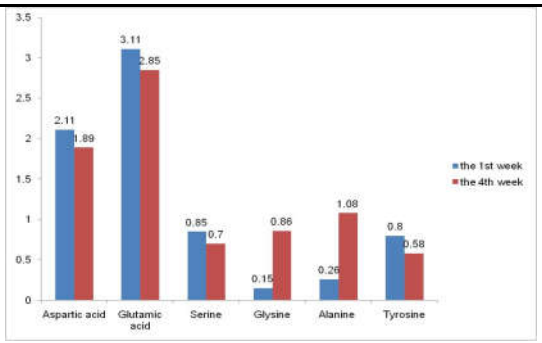

Figure 2. Profile of non-essential amino acid profiles in loin tuna which is applied by filter smoke during storage

Lysine functions as a basic ingredient of blood antibodies, strengthens the circulatory system, maintains the growth of normal cells with proline and vitamin C to form collagen tissue, decreases excess blood triglyceride levels (Harli 2008). The requirement of lycine in human body according to FAO/WHO (1985) were 0.66% (FAO/WHO in Santoso *et al*. (1996). Lysine is the skeleton in the formation of niacin (Vitamin B3), lysine deficiency can cause fatigue, difficulty in concentrating, hair loss, anemia, late growth and reproductive abnormalities (Harli 2008). Lysine content in tuna loin is high enough so that it can be used as a good source of essential amino acids to support growth. Methionine is the lowest essential amino acid found in the tuna loin treated by FS both in the 1st week storage and the 4th week storage with the number of 0.58% and 0.52% respectively. Methionine is one of two amino acids that contain the sulfuric element. Methionin serve as an element to build other amino acids like cysteine and vitamins like choline. Another importantly of methionine is to absorb the fat and cholesterol. Hence, methionine, is related to liver health, as it connected to fat synthesis (Harli, 2008). According to FAO / WHO (1985) is required 0.42% methionine in the human body (FAO / WHO 1985 in Santoso *et al*. 1996). Threonine is an important component which have an ability to maintain protein balance, convigurate the formation of collagen and elastin, increase the function of the liver, heart and system central nervous system and prevent epileptic seizures. Isoleucine is important for growing optimation, repairing damaged tissue, developing intelligence, maintaining the body's nitrogen balance, the formation of other non-essential amino acids, and the formation of hemoglobin and stabilizing blood sugar levels (Harli 2008). Leucine can stimulate brain function, increase muscle energy levels, help reduce excessive blood sugar levels, help heal bones, muscle tissue and skin (especially to accelerate post-operative wound healing) (Harli 2008). Leucine also maintain the immune system (Edison 2009). Phenylalanine is a precursor of tyrosine. Phenylalanine is needed by the thyroid gland to produce thyroxine which can prevent goiter. In addition, phenylalanine also functions to produce epinephrine and neropinephrine (Edison 2009). Methionine is important for fat metabolism, maintaining healthyness of liver, calming tense nerves, preventing the accumulation of fat in the liver and arteries, especially those that supply blood to the brain, heart and kidneys.

The human body's needs for several amino acids are as follows : threonine 0.43 %, isoleucine 0.46%, leucine 0.93%, phenylalanine 0.66%, tyrosine 0.72%, and both methionine dan sistein 0,42%. Some essential amino acids needed by the body according to FAO / WHO (1985) in Santoso *et al*. (1996), threonine 0.43, isoleucine 0.46, leucine 0.93, phenylalanine 0.66 and tyrosine 0.72, and the amount of methionine and cysteine 0.42. In the tuna loin study using gas filter smoke during the 1st week and 4th week storage for essential amino acids, such as, threonine 1.0%; 1.13%; Argin 1.29%; 1.22%; Valine 1.22%; 1.08%; phenylalanine 0.86%; 0.76%; isoleucine 1.11%; 0.99%; leucine 1.72%; 1.49%.

Based on the results of the study it appears that the average essential amino acids contained in the tuna loin applied FS have good nutrients quality because it has the availability of essential amino acids. Non-essential amino acids are amino acids that can be synthesized by the human body with other amino acids. Based on Table 1. total non-essential amino acids for tuna loin with applied by filter smoke in the first week is 8.72% and storage in the fourth week is 8.04%. According to Figure 2 the highest non-essential amino acid of tuna loin during storage in the 1st and 4th week, was glutamic acid 3.11% and 2.77% respectively. It has been reported that glutamic acid also the highest non-essential amino acid for other fish (Norouzi, 2016; ElShehawy *et al* 2016; Leiwakabessy *et al* 2016). Glutamic acid is recognized as naturally constituent component of almost all food contain high protein like meat, fish, milk and vegetable. (Apituley and Loppies, 2019). Glutamic acid can also be produced in the human body and is a very important component for human metabolism. Glutamic acid characterized by its strong taste thus stimulate the taste nerves on human tongue. The second most non-essential amino acid in tuna loin applied by filter smoke during the 1st and 4th storage was aspartic acid, which was 2.11% and 1.86% respectively, while glycine, serine and tyrosine are non-essential meet in small amount. Aspartic acid functions as supporting the liver detoxification, immune system increasing, tumor cell growth inhibition, hormone growth release, carbohydrates conversion (Harli 2008). Aspartic acid is resulted by acid hydrolysis of asparigine and glutamine. (Lehninger 1990). The number of glycine in tuna loin which treated by filter smoke during the 1st and 4th week were 0.15% and 0.86% respectively. The body need glycine for stimulates the release of growth hormones, helps wound healing, helps the development and growth of muscles, and can be used to reduce stomach acidity (Sulistyawibowo *et al* 2013). The least amino acid in tuna loin was tyrosine by the number of 0.8% and 0.58% for the 1st and 4th week storage respectivelly. Suryanti (2009) mentioned that the sequence of number of non-essential amino acids in tuna loin from the highest to the lowest were glutamic acid, aspartate acid, alanine, glycine, serine, and tyrosine. According to Suzuki (1981), the variations of chemical composition can occur in between individuals within a species and between one another parts in the body. This difference can be caused by several factors namely: age, metabolic rate, movement, food and spawning conditions.

Conclusion

There was identified 15 amino acids in tuna loin treated by filtered smoke consisting of 9 essential amino acids namely: histidine, threonine arginine, methionine, valine, penilalanin, iso leucine, leucine and lysine, and 6 non-essential amino acids namely: aspartic acid, glutamic acid, serine, glycine, alanine, tyrosine. Histidine is the highest essential amino acid found in tuna loin for the 1st week storage and lysine for the 4th week storage. The lowest essential amino acid is methionine found at the 1st and 4th week of storage ie 0.58% and 0.52% respectively. The highest non essential amino acid during storage is glutamic acid which is 3.11% and 2.77% while the lowest during storage is tyrosine 0.8% and 0.58%.

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