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Quality of *Arabushi* and *Katsuobushi* from Skipjack Tuna, Bonito, and Yellowfin Tuna

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ABSTRACT
Keumamah is traditional food in aceh (likely arabushi or katsuobushi in Japan). The aims of the research are to determine best combination of frequency and time of smoking in the production of katsuobushi using different species of fish. This research Two Stage Nested Design used (species of fish and combination of frequency and time of smoking). Species of fish studied were skipjack tuna (Katsuwonus pelamis), bonito (sarda sarda) and yellowfin tuna (Thunnus albacares) and combination of frequency and time of smoking: 10 days (1 hour/day), 5 days (2 hours/day) and 3 days (3 hours/day + 1 hour). Parameters observed in this research were amount of moisture, ash, fat, protein, N-amino, TMA-N, phenol, formaldehyde on arabushi and katsuobushi, and amino acid on the best treatment. The result this research showed species of fish had significant effect ($p<0.01$) on the moisture, ash, fat, N-amino, TMA-N, phenol and formaldehyde of katsuobushi. Combination of frequency and time of smoking in species of fish had significant effect ($p<0.01$) on the fat, protein, N-amino, TMA-N, phenol and formaldehyde of katsuobushi. The best of smoking treatment was 5 days (2 hours/day) made of skipjack tuna, with chemical composition: moisture 23.8%, ash 3.63%, fat 3.15%, protein 63.0%, N-amino 1.63%, TMA-N 3.97 mg N/100 gram, phenol 1.92%, formaldehyde 6.48 mg/100 gram and total amino acid in arabushi 49.247%db and 79.696%db on katsuobushi.
***Keywords**: keumamah, katsuobushi, arabushi*

I. INTRODUCTION

Processed fish product of traditional Indonesian food that has not received attention is keumamah (in Japan known as katsuobushi). Katsuobushi usually made from tuna (Katsuwonus pelamis), but some species of fish such as mackarel tuna (Euthynnus affinis), cigar (Auxis thazard), bonito (Sarda sarda) and tuna (Thunnus sp.) can be processed into katsuobushi. Fish processed to be katsuobushi is thick fleshy fish with fat content 1 – 3%. Katsuobushi can be used as side dishes and food flavoring [1].

Smoking on katsuobushi processing takes a long time to get a product with hard texture and low moisture content. The smoking is also intended to increase the penetration of chemical components of smoke, especially phenolic compounds. One of the factors that affect the penetration of smoke components in the material is temperature and time of smoking.

Some research on the keumamah or katsuobushi using tuna or mackarel tuna. The study on the effect of processing methods skipjack (Katsuwonus pelamis) on the nutritional value and organoleptic quality keumamah [2]. Giyatmi has done reasearch the isolation and identification of fungi in the manufacture of "ikan kayu" skipjack (Katsuwonus pelamis) [3]. Study of relative humidiy (RH) of fermentation chamber to see the effect on the growth of mold [4]. Reasearch has done a study to see the potential of tuna (Auxis thazard) as a "keumamah or arabushi" [5]. Boiling tuna using green tea extract 5% (w/v) in the processed products of smoking and katsuobushi can reduce mutagenicity [6]. Reasearch have done attempted inoculation of the fungus Aspergillus glaucus group [7]. Utilization of yellowfin tuna and bonito as katsuobushi raw materials as well as the influence of a combination of frequency and duration of smoking has not been reported.

The aims of this study to determine the quality katsuobushi bonito and yellowfin tuna than katsuobushi skipjack. Determine best combination of frequency and time of smoking in the production of katsuobushi.

II. METHOD

Research Design: This study examined the effect of species of fish and combination of frequency and time of smoking. Species of fish studied were skipjack tuna (Katsuwonus pelamis), bonito (sarda sarda) and yellowfin tuna (Thunnus albacares) and combination of frequency and time of smoking: 10 days (1 hours/day), 5 days (2 hours/day) and 3 days (3 hours/day + 1 hours). This research is laboratory experiment used Two Stage Nested

Design with three replication. The experiment was conducted in the Department of Nutrition Polytechnic of Health Malang (Microbiology, chemistry, food science and food technology laboratory), Faculty of Fisheries Processing Technology Laboratory Brawijaya University, Malang, Indonesia and Laboratory of Joint Base Airlangga University, Surabaya, Indonesia.

Materials and instruments: Skipjack (Katsuwonus pelamis), bonito (sarda sarda) and yellowfin tuna (Thunnus albacares) were used in this study was obtained from the fish auction place (TPI) Sendang Biru, South Malang. Smoke source material is coconut fiber. Other chemicals for analysis of proximate and N-amino, TMA-N and amino acids with pro analysis specification. Equipment used oven, mikrokjeldhal apparatus, Soxhlet, High Performance Liquid Chromatography (HPLC).

Processing of katsuobushi: The process of making "katsuobushi" through stages of dressing, steaming, cut into section, removal of skin and bone, and a combination of smoking and drying (Figure 1).

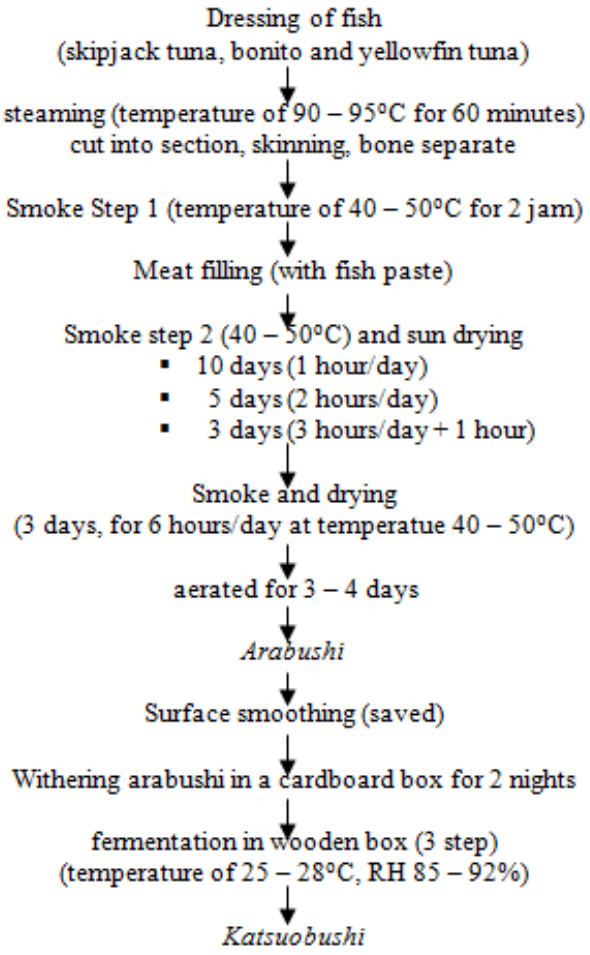

Figure 1: The process of making "katsuobushi" through stages of dressing, steaming, cut into section, removal of skin and bone, and a combination of smoking and drying.

Parameter observed in raw materials, arabushi and kastsuobushi : on the physical characteristic, pH value, TMA-N and proximate analysis: Physical characteristic include physical conditions (organoleptic), weight, thickness, standard length and total length, and proximate content (moisture, ash, fat and protein), pH and TMA-N of cakalang, bonito, and yellow fin tuna that will be used in this study. Parameter observed in arabushi and katsuobushi on the proximate content (moisture, ash, fat, protein) [8], N-amino [9], TMA-N [10], pH value [11], phenol [3], and the composition of amino acids with the Amino Acid Analyzer in the best treatment of arabushi and katsuobushi.

Amino acid analysis by HPLC [12]: 0.2 g of sample was added 10 ml of HCl 6 N, and then hydrolyzed in the oven 100°C for 24 hours. Hydrolyzate was cooled, then filtered to obtain supernatant. 10 mL of the supernatant was added to 25 mL of a solution of dryer and then dried in vacuum rotor. The treatment was repeated three times. The dried supernatant was added 30 mL of derivatization solution and left for 20 minutes, then diluted by the addition of Na-acetate pH 5.75 until 200

mL of volume. Samples preparing injected into the HPLC.
Solution of dryer made by mix of methanol, acetic acid pH 5.75 and triethanol amine (TEA) with a ratio 2 : 2 : 1. Derivatization solution was prepared by mix of 300 mL phenil Isothiocyanates (PITC), 300 mL of methanol and 40 mL of TEA .

The amino acids (%) was calculated with the formula:

$$\%\ Amino\ Acid\ = \frac{\left(\frac{AUC\ Sample}{AUC\ Standar}\right) x\ DF\ x\ MW\ x\ SD\ x\ 100}{Weight\ of\ Sample}$$

Key: DF = Dilution Factor
MW = Molecular Weight of amino acids
SD = Standard Density
AUC = Area Under Curve

Statistical analysis [13]: The data were analyzed using analysis of variance with SPSS 16, with a confidence level of 0.05. Furthermore, to determine the difference in Duncan Multiple Range Test.

III. RESULTS AND DISCUSSION

Characteristics of the Fresh Fish

Physical observations it can be seen that the three types of fish used have different sizes, where the largest fish size is yellowfin tuna, followed by skipjack tuna and bonito. Based on organoleptic assessment of fresh fish used, it can be seen that the fish used have a good freshness level (TMA-N value 4.133 - 4.533 mg N / 100 gram). Fresh fish used have a pH value from 6.03 - 6.10. This shows that the fish used are still relatively fresh (pH 6.0 - 6.5). State [14, 15] that the pH limit of fish that is still considered suitable for consumption is 6.8 and if the pH of the fish reaches 7.0 or more it is damaged
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Table 1. Physical Characteristic and Proximate Composition of the Fresh Fish

Parameters	Species of Fish		
	skipjack Tuna	Bonito	Yellow Fin Tuna
Physical characteristic			
Total length (cm)	48.30	35.25	53.25
Standar length (cm)	41.50	31.25	42.50
Thickeness (cm)	9.40	6.20	8.90
Wide (cm)	13.20	9.50	13.50
Weight (kg)	2.25	1.13	2.53
Proximate content (%)			
Moisture	72.2	74.4	73.4
Ash	1.3	1.4	1.5
Fat	1.3	1.3	1.2
Protein	26.6	26.0	25.2

TMA-N levels of fresh fish that can be accepted a maximum of 15 mg N/100 grams.
The results of proximates contents showed in Table 1. The fat content of the three types of fish used in this study according to the requirements of fish fat content to be processed into katsuobushi. Good levels of fatty fish [1] processed into katsuobushi range from 1 to 3%. Fat content very low (<1%) will produce katsuobushi that is red brown, tasteless and unfavorable flavor. Conversely, if the fish used are fat content very high (> 3%), it will produce katsuobushi which is oily, blackish brown in color, soft texture, slightly bitter taste, inadequate flavor and the resulting darker extract color.

Proximate Composition of Arabushi and Katsuobushi

Moisture and ash content of katsuobushi have increased, whereas fat and protein content of katsuobushi decreased compared to arabushi in each treatment of species of fish as well as the combination of frequency and duration of smoking in each of the same treatments. Paired t-test results showed a very significant difference ($p < 0.01$) between the water content, protein and fat of arabushi compared to katsuobushi.

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The results of Anova showed a significant defference ($\alpha<0,01$) type of fish for moistrue, ash, and fat in katsuobushi, and had no significant effect ($\alpha>0.01$) on the crude protein content of katsuobushi. Meanwhile frequency and time of smoking in the type of fish showed a significant defference ($\alpha<0,01$) on the fat and protein, and had no significant effect ($p> 0.01$) on levels water, and ash. Proximate composition of the arabushi showed on Table 2 and katsuobushi showed on Table 3

Table 2. Proximate composition of the *arabushi*

frequency and time of smoking	species of fish		
	skipjack Tuna	Bonito	Yellow Fin Tuna
	moisture		
10 days (1 hour/day)	15.4 ± 0.40	13.3 ± 0.19	15.3 ± 0.60
5 days (2 hours/day)	15.7 ± 0.19	14.1 ± 0.05	16.1 ± 0.05
3 days (3 hours/day + 1 hour)	16.1 ± 0.09	14.4 ± 0.01	16.7 ± 0.34
	ash		
10 days (1 hour/day)	3.5 ± 0.01	3.9 ± 0.14	4.0 ± 0.03
5 days (2 hours/day)	3.5 ± 0.02	3.8 ± 0.15	4 ± 0.03
3 days (3 hours/day + 1 hour)	3.4 ± 0.13	3.8 ± 0.10	3.8 ± 0.17
	protein		
10 days (1 hour/day)	67.4 ± 0.54	69.0 ± 1.43	69.1 ± 0.55
5 days (2 hours/day)	71.4 ± 0.68	71.3 ± 0.68	68.5 ± 0.52
3 days (3 hours/day + 1 hour)	70.8 ± 0.58	71.9 ± 0.64	67.9 ± 0.3
	fat		
10 days (1 hour/day)	3.6 ± 0.04	4.1 ± 0.05	3.1 ± 0.03
5 days (2 hours/day)	3.9 ± 0.04	4.3 ± 0.05	3.1 ± 0.01
3 days (3 hours/day + 1 hour)	3.8 ± 0.04	4.6 ± 0.04	3.2 ± 0.05

Table 3. Proximate composition of the *katsuobushi*

frequency and time of smoking	species of fish		
	skipjack Tuna	Bonito	Yellow Fin Tuna
	moisture		
10 days (1 hour/day)	23.2 ± 1.79	20.3 ± 0.15	23.9 ± 0.77
5 days (2 hours/day)	23.8 ± 0.59	20.8 ± 0.37	23.9 ± 1.14
3 days (3 hours/day + 1 hour)	23.4 ± 1.14	19.4 ± 1.19	24.4 ± 0.19
	ash		
10 days (1 hour/day)	3.08 ± 0.47	4.09 ± 0.06	4.01 ± 0.77
5 days (2 hours/day)	3.63 ± 0.33	4.11 ± 0.10	4.36 ± 0.17
3 days (3 hours/day + 1 hour)	3.29 ± 0.38	3.95 ± 0.10	3.62 ± 0.55
	protein		
10 days (1 hour/day)	63.5 ± 1.67	61.8 ± 1.12	64.4 ± 0.62
5 days (2 hours/day)	63.0 ± 0.05	62.9 ± 0.17	61.6 ± 1.01
3 days (3 hours/day + 1 hour)	62.9 ± 0.49	63.8 ± 0.57	63.3 ± 0.84
	fat		
10 days (1 hour/day)	3.13 ± 0.01	3.32 ± 0.01	2.97 ± 0.02
5 days (2 hours/day)	3.15 ± 0.05	3.03 ± 0.03	3.01 ± 0.00
3 days (3 hours/day + 1 hour)	3.33 ± 0.02	3.54 ± 0.04	3.04 ± 0.02

The moisture content of katsuobushi bonito is significantly different from the water content of skipjack katsuobushi and yellowfin tuna katsuobushi, while the water content of skipjack tuna katsuobushi is not significantly different from the water content of yellowfin tuna katsuobushi. The difference is caused by differences in the size of the fish used. Skipjack tuna and yellowfin tuna have a larger size (length, width, thickness and weight) compared to bonito. This has an impact on the size of the surface area of the fish.

The surface area of the material determines the distance and transfer time of hot air from the surface into the material, as well as the time and distance that must be transfer by the mass of water from inside to the surface of the material. This condition causes the drying rate to be higher in smaller sized materials compared to larger sized materials with smaller surface area. The rate of mass transfer of water from inside to outside food during drying is influenced by the surface area of the material, temperature and drying time, air flow velocity, air humidity and atmospheric pressure [16].

Decrease of protein content in each type of treatment is thought to be caused by the growth of proteolytic molds, such as Aspergillus and Rhizopus. That mold Rhizopus sp is able to produce protease enzymes. Rhizopus oligosporus produces the highest activity protease enzyme, Rhizopus stolonifer is lower, and the lowest is Rhizopus oryzae and Rhizopus arrhizus [17].

The fermentation stage cause a decrease protein content in katsuobushi, where the amount of reduction was 0.98% in katsuobushi from skipjack tuna and 5.07% in katsuobushi from tuna mackarel [5]. The combination treatment of frequency and duration of smoking affected the growth of molds during arabushi fermentation. This is the cause of the difference in katsuobushi protein content between treatments. In katsuobushi bonito mold growth is more dense, which will certainly affect the amount of protease enzymes produced. The activity of the protease enzyme which hydrolyzes proteins into peptides and amino acids followed by the deamination process produces ammonia. This causes nitrogen to disappear (evaporate) with ammonia.

Decreased of fat content in katsuobushi can be caused because during the fermentation process of arabushi to katsuobushi, the surface of arabushi is covered with lipolytic molds, namely Aspergillus repens, Aspergillus chevalieri, and Rhizopus oligosporus and Rhizopus oryzae. That molds which generally grow during fermentation of arabushi are lipolytic molds, although at a weak level [3]. One of the goals of mold growth (fermentation) in making katsuobushi is to reduce the fat content in the final product [18].

The three types of katsuobushi it can be seen that the lowest fat content is katsuobushi from yellow fin tuna, followed by skipjack and bonito. This is because the fat content in fresh yellow fin tuna is indeed lower than fresh tuna and bonito. Intensity of mold growth in arabushi during fermentation also affects the katsuobushi fat content. The highest intensity of mold growth in arabushi with 5 days (2 hours/day) smoking, followed by 10 days (1 hour/day) and 3 days (3 hours/day + 1 hour). During the fermentation [19] of lipolytic molds will break down fats into fatty acids and flavor compounds, such as aldehydes, alcohols and methyl ketones which can reduce total fat content.

Heat, acidity or base high treatment and activity of protease enzymes can be hydrolyzed proteins to become free amino acids. In the advanced stage the process of protein hydrolysis can be followed by deamination of free amino acids into ammonia, so that reducing the amount of amino nitrogen [20]

The steaming step of the fish at the same temperature and time (90 to 95oC, for 60 minutes) gives different levels of hydrolysis in each species of fish. In this condition the size and structure of fish muscle tissue affect the strength and speed of hydrolysis. The smallest bonito,

The effect of combination of frequency and time of smoking on each species of fish is evident, where the 5-day (2 hours/day) smoking treatment has the highest N-amino content. This is thought to be related to the intensity of proteolytic molds that grow on the surface of the arabushi during the fermentation stage. In Arabushi with 5 days (2 hours/day) smoking treatment, molds have begun to grow at stage I fermentation with the highest intensity followed by arabushi from skipjack tuna, while arabushi from yellow fin tuna on stage I fermentation has not been overgrown with mold. That amino acids [21] are one of the compounds derived from protein hydrolysis by the activity of protease enzymes. Protease enzymes play a role in protein hydrolysis to produce peptons, peptides and amino acids followed by deamination

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N-amino and TMA-N composition of arabushi and katsuobushi

Table 4. N-amino, TMA-N, Phenol and formaldehyde composition of the *arabushi*

frequency and time of smoking	species of fish		
	skipjack Tuna	Bonito	Yellow Fin Tuna
	N-amino		
10 days (1 hour/day)	1.07 ± 0.09	0.76 ± 0.03	1.00 ± 0.06
5 days (2 hours/day)	1.08 ± 0.03	0.75 ± 0.03	1.06 ± 0.02
3 days (3 hours/day + 1 hour)	1.20 ± 0.20	0.69 ± 0.04	1.05 ± 0.04
	TMA-N		
10 days (1 hour/day)	6.80 ± 0,80	7.87 ± 1.51	5.47 ± 1.62
5 days (2 hours/day)	6.13 ± 0.61	7.60 ± 0.40	5.47 ± 1.67
3 days (3 hours/day + 1 hour)	5.93 ± 0.64	7.13 ± 0.42	4.67 ± 1.29
	phenol		
10 days (1 hour/day)	2.70 ± 0.07	3.91 ± 0.03	2.52 ± 0.11
5 days (2 hours/day)	2.70 ± 0.13	3.97 ± 0.05	2.54 ± 0.03
3 days (3 hours/day + 1 hour)	2.39 ± 0.06	4.21 ± 0.01	2.33 ± 0.01
	formaldehyde		
10 days (1 hour/day)	7.39 ± 0.19	8.04 ± 0.20	6.70 ± 0.19
5 days (2 hours/day)	7.54 ± 0.11	8.34 ± 0.25	6.95 ± 0.17
3 days (3 hours/day + 1 hour)	7.86 ± 0.11	8.93 ± 0.21	7.49 ± 0.16

Table 5. N-amino, TMA-N, Phenol and formaldehyde composition of the *katsuobushi*

frequency and time of smoking	species of fish		
	skipjack Tuna	Bonito	Yellow Fin Tuna
	N-amino		
10 days (1 hour/day)	1.43 ± 0.02	1.21 ± 0.01	1.16 ± 0.03
5 days (2 hours/day)	1.63 ± 0.07	1.43 ± 0.19	1.39 ± 0.21
3 days (3 hours/day + 1 hour)	1.23 ± 0.05	1.14 ± 0.02	1,13 ± 0.06
	TMA-N		
10 days (1 hour/day)	6.07 ± 0.42	5.47 ± 0.31	4.77 ± 0.67
5 days (2 hours/day)	3.97 ± 0.32	5.43 ± 0.49	4.97 ± 0.21
3 days (3 hours/day + 1 hour)	4.17 ± 0.25	5.83 ± 0.21	3.33 ± 0.15
	phenol		
10 days (1 hour/day)	2.06 ± 0.03	2.82 ± 0.03	2.10 ± 0.01
5 days (2 hours/day)	1.92 ± 0.07	2.76 ± 0.06	1.90 ± 0.02
3 days (3 hours/day + 1 hour)	1.71 ± 0.01	3.10 ± 0.02	1.78 ± 0.05
	formaldehyde		
10 days (1 hour/day)	52.22 ± 0.73	69.96 ± 0.94	59.48 ± 1.09
5 days (2 hours/day)	64.82 ± 2.26	77.66 ± 1.30	64.72 ± 1.10
3 days (3 hours/day + 1 hour)	63.21 ± 1.02	84.48 ± 3.84	67.17 ± 1.86

Drying process between the fermentation time causes a decrease in TMA-N content in katsuobushi. Methyl groups [20] have volatile properties. The decrease in TMA-N levels could possibly be caused by mold activity during fermentation which would break down the amine compound into volatile ammonia. That microorganisms [22, 23] need nitrogen to process their growth. Nitrogen can be obtained from organic materials, such as ammonium (NH4), nitrate (NO3) or organic matter in the form of amino acids, peptides and proteins.

The Best Treatment

The results of calculations using the effectiveness index method [24,25] obtained the best treatment katsuobushi according to fish type is katsuobushi which received 5 days (2 hours/day) smoking treatment for skipjack tuna and bonito and smoking treatment 10 days (1 hour/day) for yellow fin tuna. Overall the best katsuobushi is made from skipjack tuna which received 5 days (2 hours/day) smoking treatment with an effectiveness index value of 0.564.

Amino Acids Composition Arabushi dan katsuobushi the Best Treatment

Amino acid composition of arabushi and katsuobushi the best treatment showed in Table 5. Total amino acids After three stages of fermentation, each of 13 days, 7 days and 7 days showed an increase levels in all types of amino acids. This is consistent with the results of measurements of N-amino levels, where the levels of N-amino katsuobushi (after fermentation) are higher than the levels of N-amino arabushi (before fermentation).

Amino acid is one of the non volatile compounds from neutral fraction which together with the volatile compound phenol group from the weak acid fraction will form a specific flavor that is dominant in katsuobushi [26].

Composition amino acids of the katsuobushi and arabushi shows that the glutamic amino acid content is quite high, which is 13.798%. Glutamate levels increased 58.58% compared to the levels of glutamate of arabushi, which is 8.701%. Lysine levels in katsuobushi, which is 7.076% increased 58.99% compared to arabushi lysine levels, which is 4.445%. Histidine levels in katsuobushi, which is 4.568% increased 57.3% compared to arabushi histidine levels, which is 2.904%.

There is a synergistic effect between several amino acids in increasing umami taste. The mixture of amino acids include IMP and GMP with aspartate and / or glutamate in 1% NaCl solution. Glutamate-sodium mixture (monosodium glutamate) will contribute 71.4% to the umami taste of the total basic taste (sweeteness, sourness, bitterness, saltiness and umami). In the singular form, glutamate only contributed 24.8% to umami taste and the highest contribution to sourness, which was 60.0% [27,28]

The largest single amino acid contribution to umami taste was glutamate (24.8%), followed by serine (19.1%), methionine (16.6%), alanine (13.1%) and histidine (11.5) %). Of the five types of amino acids serine and alanine gave a dominant sweet taste (64.7% and 75.1%), glutamate gave a dominant acid taste (60.0%), whereas histidine and methionine predominantly gave a bitter taste (64, 1% and 61.6%

Tabel 6. Komposisi asam amino *arabushi* dan *katsuobushi* (% dry base)

No.	Amino acids	Arabushi	Katsuobushi	Increase (%)
1.	Aspartat	4.884	7.713	57.9
2.	Threonin	2.413	3.779	56.6
3.	Serin	1.994	3.099	55.4
4.	**Glutamat**	**8.701**	**13.798**	**58.6**
5.	Glysin	2.462	3.689	49.8
6.	Alanin	3.051	5.049	65.5
7.	Cystein	0.252	0.646	156.3
8.	Valin*	2.755	4.374	58.8
9.	**Metionin***	**0.123**	**1.888**	**1435.0**
10.	Isoleusin*	2.385	3.929	64.7
11.	Leusin*	4.045	6.695	65.5
12.	Tyrosin*	2.251	2.881	28.0
13.	Phenilalanin*	2.085	3.471	66.5
14.	**Lysin***	**4.445**	**7.067**	**59.0**
15.	**Histidin***	**2.904**	**4.568**	**57.3**
16.	Arginin*	2.922	4.651	59.2
17.	Prolin	1.575	2.399	52.3
	Total asam amino	**49.247**	**79.696**	**61.8**

IV. CONCLUSION

Species of fish had significant effect ($p<0,01$) on the moisture, ash, fat, N-amino, TMA-N, phenol and formaldehyde of katsuobushi. Combination of frequency and time of smoking in species of fish had significant effect ($p<0,01$) on the fat, protein, N-amino, TMA-N, phenol and formaldehyde of katsuobushi. The best of smoking treatment was 5 days (2 hours/day) made of skipjack tuna, with chemical composition: moisture 23.8%, ash 3.63%, fat 3.15%, protein 63.0%, N-amino 1.63%, TMA-N 3.97 mg N/100 gram, phenol 1.92%, formaldehyde 6.48 mg/100 gram and total amino acid in arabushi 49.247%db and 79,696%db on katsuobushi.

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