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Amino acid and fatty acid compositions of Rusip from fermented Anchovy fish (*Stolephorussp*)

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Abstract. Rusip is a typical food of Bangka Belitung Indonesia made from fermented anchovy. This study aims to determine the properties of chemistry, microbiology, composition of amino acids and fatty acids from fermented fish spontaneously and non spontaneously. Spontaneous rusip treatment is done by anchovy fish (*Stolephorus*sp) after cleaning and added salt 25% (w/w) and palm sugar 10% (w/w). While, non-spontaneous rusip is done by adding a culture mixture of *Streptococcus, Leuconostoc,* and *Lactobacillus* bacteria 2% (w/v). The materials are then incubated for 2 weeks. The data obtained were then performed t-test at the level of 5%. Spontaneous and non-spontaneous rusip fermentation process showed significant differences in total acid, reducing sugar, salt content, TVN, total lactic acid bacteria, total mold, and total microbial. The dominant amino acid content of spontaneous and non-spontaneous rusip are glutamic acid and aspartic acid, while the dominant fatty acids in spontaneous and non-spontaneous rusip are docosahexaenoic acid, palmitic acid, oleic acid, arachidonic acid, stearic acid, eicosapentaenoic acid, palmitoleic acid, and myristic acid.

1. Introduction

Rusip is typical of Bangka Belitung Indonesian food made from fermented anchovy [18] [1]. Besides fish, other ingredients are added in the manufacture of salt rusipare 25% and 10% of palm sugar, then fermented for 1-2 weeks. Rusip have a light brown color to dark gray, sweet, sour and salty, typical aroma of fish, and generally consumed as a condiment or mixed dishes [18].[17]states that the value of TVN rusip still high, so it needs further in detail processing with the addition of lactic acid bacteria culture. The results showed changes in microbiological and chemical properties rusip during the fermentation process. The fermentation process is non-spontaneous rusip have chemical and microbiological properties better than spontaneous fermentation [19]. Changes in chemistry, microbiology, amino acids, fatty acids, as well as sensory may occur during the process of fermentation of fish due to processing and differential treatment[3],[8],[15],[16],[21], [33],[35].Lactic acid bacteria have a big impact on the nutritional quality and shelf life of the products of fermentation [2].This study aims to determine the nature of chemistry, microbiology, as well as the composition of amino acids and fatty acids rusip from fermented anchovy spontaneous and non-spontaneous.

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2. Materials and methods

Spontaneous rusip processing is done by anchovy *(Stolephorussp)* after cleaning and then added salt as much as 25% (w / w) and palm sugar 10% (w / w). While the non-spontaneous rusip done by adding mixed cultures of bacteria *Streptococcus, Leuconostoc,* and *Lactobacillus* as much as 2% (w/v). The next ingredient incubated for 2 weeks [19].

Observations chemical and microbiological include: total acid [4], protein content [4], fat [4], reduced sugar [9], the water content [4], salinity [7], pH [4], TVN [4], total lactic acid bacteria [11], total microbial [11], total mold [11], the amino acid composition (HPLC), and the fatty acid composition (GC-MS). The data were then performed t test at 5% level.

3. Results and discussion

Spontaneous and non-spontaneous rusip production was performed with the same incubation period of 2 weeksThe observation of chemical and microbiological properties of spontaneous and non-spontaneous rusip can be seen in Table 1.

Table 1. Characteristics of chemical and microbiological properties of Rusip

parameters	Rusip	
	Spontaneous	Non spontaneous
Total acid (%)	2.28[a]	3.60[b]
protein content (%)	14,45[a]	13,64[a]
Fat content (%)	0,38[a]	0,44[a]
Sugar reduction (%)	6.42[a]	5.01[b]
Moisture content (%)	66,52[a]	67,27[a]
Salinity (%)	24,94[a]	19,49[b]
pH	5,61[a]	5,74[a]
TVN (mg N / 100 g)	31.76[a]	20.19[b]
Total lactic acid bacteria (log cfu /g)	9.66[a]	12.09[b]
Total mold (log cfu /g)	9.66[a]	5.81[b]
Total microbe (log cfu /g)	9.84[a]	8.42[a]

Description: Figures in row followed by the same letters mean no significant difference in the level of 5%

The results showed that the protein content of spontaneous rusip(14.45%) and non-spontaneous (13.64%) more than in the previous study by 12.81% [15]. Table 1 shows the differences in the characteristics of the spontaneous and non-spontaneous rusip due to differences in the total amount of lactic acid bacteria in rusip. Rusip non spontaneous containing lactic acid bacteria 12:09 log cfu / g higher than the spontaneous rusip 9.66 log cfu/g. This is because the process of making non spontaneous rusip added mixed cultures of lactic acid bacteria *Streptococcus*,Leuconostoc,and*Lactobacillus.*Thus, the lactic acid bacteria dominate the growth during the fermentation process rusip[19]. This is in line with research [1] which states that the fermentation rusip with the addition of mixed cultures of lactic acid bacteria produce a total lactic acid bacteria higher compared to single culture. the high total lactic acid bacteria fermentation rusip give different characteristics of the resulting product as the value of total acid, salinity, TVN, total fungi, and total microbes.

Rusip non spontaneous containing acid total 3.60% higher than rusip spontaneous 2.28%. This is because the mixed culture of lactic acid bacteria added to the fermentation process can increase the growth rate and produce lactic acid through heterofermentative and homofermentative processes [20]One of the lactic acid bacteria added to the process of making rusip is Leuconostocsp which is a heterofermentative group of bacteria [23]. Leuconostocsp bacteria can grow well for 15 days of fermentation process [22]. Addition of lactic acid bacteria culture to the sindol which is a fish

fermentation product from India gives changes of organoleptic, physicochemical, and microbiological properties of the resulting product [5].
Non-spontaneous rusip has a salt content 19.49% lower than spontaneous rusip 24.94%. The dominant growth of lactic acid bacteria since the beginning of the fermentation process is able to suppress the growth of bacterial decomposition and pathogens [30].Thus, the use of salt by lactic acid bacteria in spontaneous rusip more than spontaneous rusip[20].The high lactic acid bacteria in non spontaneousrusip also affects the decrease of TVN, total mold, and total microbial value[19] [20].The addition of cultures of lactic acid bacteria to fermentation products will produce metabolite products that are antimicrobial, increase sensory, nutrient, and shelf life of the resulting product[2], [31], [32].

Table 2.Amino acid compositions ofRusip

Amino acids (%)	Rusip	
	Spontaneous	Non spontaneous
aspartic acid	1,39 a	1.41 a
glutamateacid	2,07 a	2.06 a
serine	0,56 a	0 , 56 a
histidine	0,31 a	0.28 a
glycine	0,57 a	0.61 a
threonine	0.55 a	0.51 a
arginine	0.55 a	0.53 a
alanine	0.91 a	0.90 a
tyrosine	0.43 a	0.45 a
methionine	0.33 a	0.07 b
valine	0.66 a	0.66 a

Description: Figures in row followed by the same letters mean no significant difference in the level of 5%

Table 2 shows that the amino acid composition of spontaneous and non spontaneousrusip is complete both essential and non essential amino acids. The results showed that amino acid composition between non spontaneous and spontaneous rusip was not significantly except for methionine and lysine. Dominant amino acid in rusip is glutamate acid and aspartate acid. [15] mentioned that glutamate acid, aspartate acid, and lysine are the dominant amino acids in the rusip with higher protein digestibility values than budu.[12]stated that the addition of mixed cultures to the fish fermentation can increase the amino acid composition. The composition of amino acids is also similar to other fish fermentation products such as oyster sauce[3], fish sauce [14] and[6], sauce oyster [13],fermented shrimp [10],budu and Rusip[15].
The dominant amino acid composition in fish fermentation product is glutamate acid. The difference in the number of each amino acid is caused by the difference in the free amino acid balance produced by autolysis and microbial activity [14]. Amino acids significantly affect the taste of Yu-lu fish fermentation products [25],[14]. Glutamate acid gives the aroma of meaty[14]. Amino acids glycine, alanine, serine, and threonine give a sweet taste, while valine, phenylalanine, and histidine give a bitter taste [24].[27]states that glutamic acid is closely related to "umami", which is produced as a relatively stable amino acid in the absence of secondary decomposition during fermentation.

Table 3.Fatty acidcompositions of Rusip

Fatty acids (%)	Rusip	
	Spontaneous	Non spontaneous
caprylic acid, C8: 0	0.05 a	0.05 a
capric acid, C10: 0	0.03 a	0.04 a
lauric acid, C12: 0	0.34 a	0.30 a
acid tridecanoat, C13: 0	0.11 a	0.11 a
acid myristate, C14: 0	3.21 a	2.87 a
acid pentadekanoat, C15: 0	0.61 a	0.56 a
acid palmitate, C16: 0	10.95 a	9.62 a
acids palmitoleic, C16: 1	3.26 a	2.89 a
acid heptadekanoat, C17: 1	0.82 a	0.71 a
acid cis-10-heptadecanoat, C17: 1	0.16 a	0.15 a
stearic acid, C18: 0	4.12 a	3.52 a
acid elaidic, C18: 2n6c	0.08 a	0.06 a
oleic acid, C18: 1n9c	4.66 a	4 , 15 a
linoleic acid, C18: 2n6c	0.67 a	0.61 a
arakidat acids, C20: 0	0.31 a	0.26 a
g-linolenic acid, C18: 3n6	0.15 a	0.13 a
acid cis-11-eicosenoat, C20: 1	0.08 a	0.07 a
acids linolenic, C18: 3n3	0 , 40 a	0.25 a
heneicosanoat acids, C21: 0	0.09 a	0.08 a
acid cis-11,14- eicosedienoat, C20: 2	0.10 a	0.10 a
behenic acid, C22: 0	0.43 a	0.37 a
acid cis-8,11,14-eicosetrienoat, C20: 3n6	0.13 a	0.13 a
erucat acid, C22: 1N9	0.02 a	0.03 a
acid cis-11,14,17-eicosetrienoat, C20: 3n3	0.04 a	0.05 a
arachidonic acid, C20:4n6,	4.53 a	4.01 a
tricosanoat acid C23: 0	0.08 a	0.12 a
acid lignocerat, C24: 0	0.79 a	0.74 a
acid cis-5,8,11,14,17-eicosapentaenoic, C20: 5n3	4.05 a	3.37 a
nervonat acid, C24: 1	0.23 a	0.19 a
cis-4,7,10,13,16 Acid, 19-docosahexaenoat, C22: 6n3	16.62 a	14.77 b

Table 3 shows that fatty acids dominant in rusip are docosahexaenoic acid, palmitic acid, oleic acid, arachidonic acid, stearic acid, eicosapentaenoate, palmitoleic acid, and myristic acid. The results showed that the composition of spontaneous and non-spontaneous fatty acids was not significantly different except docosahexaenoic acid. The content of docosahexaenoic acid in spontaneous rusip is 16,62% higher than non spontaneous 14,77%. The high content of docosahexaenoic acid causes a distinctive aroma in fish sauce[6].[34] mentioned that the content of docosahexaenoic acid in the bekasem from anchovy fish is lower than the fresh anchovy. The fatty acid docosahexaenoate decreases after fermentation by addition of bacterial culture of lactic acid. This can happen because of the decrease in salt concentration in the rusip. Non-spontaneous rusip has a lower salinity than spontaneous rusip due to the growth of lactic acid bacteria utilizing salt from 25% to 19.49[20].

The fatty acid with one double bond (MUFA) or more than one double bond (PUFA) is an essential fatty acid commonly found in fish and is needed by the body [29].[26]states that free radicals can cause cellular damage in the body, so antioxidant compounds are needed. Antioxidant compounds can prevent damage by inhibiting the production of major catalysts in lipid peroxidation. The Maillard Reaction products (MRPs) formed during the fermentation process can increase antioxidant activity [28].[3]states that shrimp paste fermented with 25% salt addition for 36 days indicates antioxidant activity. Rusip is a fermented product of fish fyang is also likely to show the antioxidant activity obtained from fatty acid content.

4. Conclusions

The fermentation process rusip spontaneous and non-spontaneous show a real difference to the total acid, reducing sugar, salt content, TVN, total lactic acid bacteria, total fungi, and total microbes. The amino acid content dominant in rusip spontaneous and non-spontaneous are glutamic acid and aspartic acid, while the fatty acids predominantly on rusip spontaneous and non-spontaneous are docosahexaenoatacid, palmitic acid, asam oleic asamarachidonic, stearic acid, eicosapentaenoic acid, palmitoleic acid and myristic acid.

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