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The Formulation And Characterization Of Hydrolyzate Base Sauce Produce By Enzymatic Hydrolysis From Inferior Fish

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Abstract: Food companies are demanding food industry to invent food innovation from quality natural ingredients. One of the top quality natural ingredients is inferior fish (Tonguefish, Cardinalfish, and Flathead). The inferior fish is utilized for the manufacture of hydrolyzate protein enzymatic hydrolysis processed using enzyme protease and papain calotropis gigantea from the plant. Hydrolyzate protein of the inferior fish can be used as raw material of natural seasoning without any mixture of synthetic amino acids. In manufacturing of the fish sauce needs to be added thickening agents such as maltodextrin and CMC. The addition of thickening agents contribute in the formation characteristics of good fish sauce and preferred. The purpose of this research is to determine characteristics of the derived fish sauce as well as comparison of the right maltodextrin or CMC as thickener from the inferior fish as enzymatic hydrolyzate. This research result showed the viscosity of 9,79 Pa.S. Maillard products 0.43, AU, brightness 44,38, of dissolved proteins 8,425mg/ml, ash levels 2.68%, protein levels 7,52%, organoleptic color 3,19; the scent of 3,46; the taste 3,00 – 3,04; viscosity of 3,27; and overall 3, 23 (rather like to like). The result of effectiveness test of the highest or best value is the treatment S. B (1%CMC) with the value of 0.772.
Keyword : inferior fish; hydrolysis; thickener (maltodekstrin and CMC).

Introduction

The development of food industry demanding the food companies to invent a product innovation from the quality natural ingredients. Natural ingredients with the potential to be developed is inferior fish which numbers is very abudant, but the utilization is not optimal. This inferior fish is utilized to manufacturing protein hydrolyzate with using hydrolysis process enzymatically. Hidrolysis product of the extract material containing protein is called as protein hydrolyzate could potentially be used as seasoning. During hydrolysis process, protein will undero changes so the protein hydrolyzate could be applicated for certain purpose, besides the flavor in food in the form of volatile components[1]. During processing, various volatile components will be damaged and disappear, but some of the precursor compounds remain intact in the material. This precursor compounds could be hydrolyzed enzymatically to generate the flavor, this process is called *flavourase*[2.] Utilization Utilization of protein hydrolyzate is relatively cheap than mixture of amino acids synthesis[3].

This hydrolyzed of inferior fish consists of 3 kinds of fishes namely Tonguefish, Cardinalfish, and Flathead which could be used as raw material of fish sauce manufacturing which will be applied on the food[4]. In this case inferior fish could be used as raw material of fish sauce manufacturing which will be applied on the food. Sauce manufacturing mainly needed additional material for sauce thickening agent. The use of this thickening agent needs to be considered as not every agent compatible to be used as sauce thickening agent. Besides, the addition of thickening agent has maximal limit of usage. Thickening agent serves to repairing texture, stabilizing emulsion and enhancing viscosity on the sauce[5]. Thickening agent which mainly used is CMC and Maltodextrin. CMC (*Carboxymethyl Cellulose*) is hydrophilic colloid. CMC is one of natural

hydrophilic which has been modified and anionic polyelectrolyte. Hydrophilic or hydrophilic colloid is an important addictive component in the food industry as its ability to change the properties functionality of food product. Hydrophilic used for suspense stability. Maltodextrin as starch hydrolysis product contain α-D-glucose which mostly related through binding α 1,4 glycosidic with DE (*Dextrose Equivalent*) less than 20[6]. This maltodextrin could be used as thickening agent and also as emulsifier and could enhance agent viscosity material[7].

This research focused in the formulation of fish sauce using the best product of wet hydrolyzate of inferior fish as the main raw material using enzyme protease and papain with comparison 70%:30%[4]. Characteristic of fish sauce produced by enzymatic technique mixed with Meltodextrin and CMC as sauce thickening agents.

Materials and Methods

Raw materials used this experiment used of 3 types of fish i.e tonguefish (*Cynoglussus lingua*), Cardinalfish (*Apogon albimaculosus*) and flathead (*Platycephalidae cymbacephalus*), Biduri protease and papain, maltodekstrin, CMC, brown sugar, glucose, extract of fermented cassava, deaf, and commercial sauce (Saori). Chemicals used aquades, selenium, H_2SO_4, 0,2 N NaOH, psosphate buffer pH 7, Mix – Lowry (Na_2CO_3 anhydrous $CuSO_4$), Follin, tyrosine standart, BSA standarts, boric acids. Tools used include: stainless blender, centrifugeYenaco YC-1180 models and the tube, Roy Spectronic 21D Melton and cuvet, Jen Way-type 3320 of pH meter(Germany), magnetic stirer Stuart Scientific and stonestirer, vortex Thermolynetype 16700, refrigerator, stopwatch, oswald viscometer, waterbath GFL 1083, Ohaus analytical balance, electric heating Gerhardt, spatula, vacuum oven, vortex Maxi Max Type 16700, 80mesh of sieve, distillates, biuret, film bottles, thenequipment for sensory analysis such as trays, plastic cups,spoons, testing form.

Production Hydrolyzate of Inferior Fish

At this stage of the preparation begins by separating the inferior fish bone from flesh so the resulting pure meat as much as 30 grams tonguefish, much as 30 grams Cardinalfish, much as 40 grams flathead. Each of these fish total weight of fish which is 100 grams of inferior kind of fish then steamed for 10 minutes after it is destroyed using the blender with water and comparison of 2: 1 (weight/weight). The resulting inferior fish suspension added a mixture of enzymes protease and papain calotropis gigantea by comparison according to the previous research was the best treatment (70% B: 30% P). The concentration of the enzyme namely 0.15% (% of the weight of fish meat suspension inferior)[4]. Then the pH 7 pH to be regulated by adding NaOH 0,2N and hydrolyzed in the waterbath temperature 55 ° C for 90 minutes, so it simmer for 10 minutes to inactivation of the enzyme.

Fish Sauce Production

Wet hydrolyzate of the inferior fish meat sauce made by adding herbs such as brown sugar (30%), glucose (10%), extract of fermented cassava (5%), and deaf (1%) of the weight of the wet flesh of hydrolyzate fish inferior. After it's boiled for 15 minutes and do the filtering. Then the wet fish inferior hydrolyzate added maltodekstrin 1%; CMC 1%; maltodekstrin (0.5%) + CMC (0.5%) and without the addition of thickening (control), followed by boiling it to boil for 30 minutes. Then filtered back and brings the inferior fish sauce. As for the formulation of the addition of thickening on the creation of inferior fish sauce is as follows:

Table 1. Formulation Addition Seasoning and Thickening Agent

Treatment	Inferior Fish Sauce Seasoning (%)				Addition of Thickening Agent
	Brown Sugar	Glucose	Extract of fermented cassava	deaf	
S.A.	30	10	5	1	1% Maltodextrin
S.B.	30	10	5	1	1% CMC
S.C.	30	10	5	1	0,5% Maltodextrin + 0,5%CMC
S.D	30	10	5	1	Without addition of thickening agent

Result

Viscosity

Viscosity of inferior fish sauce from the raw material of hydrolyzate inferior fish with addition various kinds and procentage of thickening agent ranged between 6,17 – 9,79 Pa.S. Histogram of viscosity value can be seen in Figure 1.

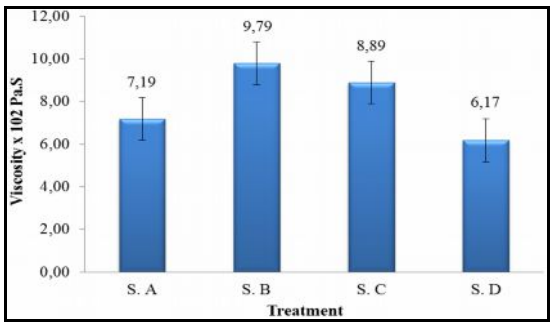

Figure 1. Histogram of Inferior Fish Sauce Viscosity

Maillard Product

Observation result of Maillard sauce product from the raw material of hydrolyzate inferior fish with addition various kinds and procentage of thickening agent ranged between 0,27 – 0,43. Histogram of absorbance value maillard product can be seen in Figure 2.

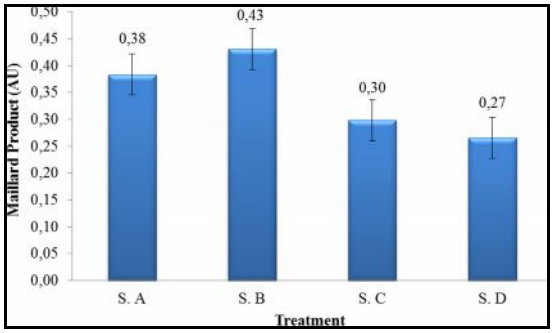

Figure 2. Histogram of Inferior Fish Maillard Product

Color

Observation result of color produced by sauce from the raw material of hydrolyzate inferior fish with addition various kinds and procentage of thickening agent ranged between 41,31 – 44,38. Histogram of color value from the inferior fish sauce can be seen in Figure 3.

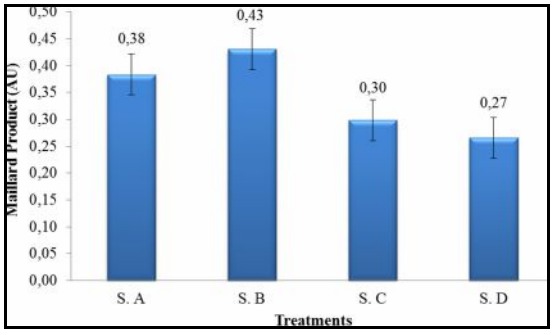

Figure 3. Histogram of Inferior Fish Color

Soluble Protein

Observation result of sauce soluble protein from the raw material of hydrolyzate inferior fish with addition various kinds and procentage of thickening agent ranged between 7,984 – 8,425 (mg/ml). Histogram of soluble protein value from the inferior fish sauce can be seen in Figure 4.

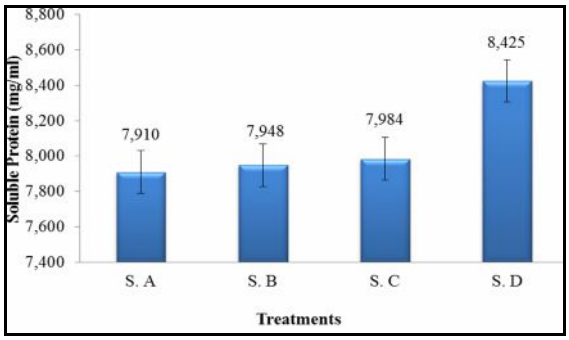

Figure 4. Histogram of Inferior Fish Sauce Soluble Protein

Ash Content

Observation result of ash content of sauce from the raw material of hydrolyzate inferior fish with addition various kinds and procentage of agent without addition of thickening agent ranged between 1,93 – 2,68 (%). Histogram of ash content from the inferior fish sauce can be seen in Figure 5.

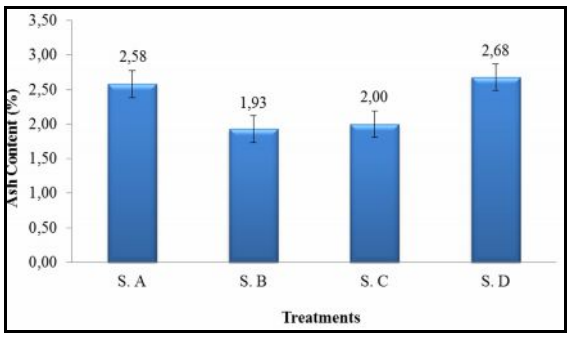

Figure 5. Histogram of Inferior Fish Sauce Ash Content

Protein Content

Observation result of protein content of sauce from the raw material of hydrolyzate inferior fish with addition various kinds and procentage of thickening agent ranged between 7,21 – 7,52 (%). Histogram of protein content from the inferior fish sauce can be seen in Figure 6.

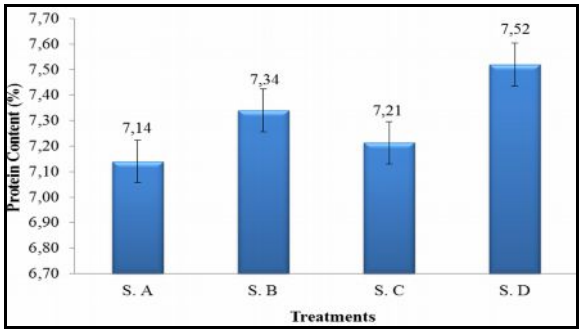

Figure 6. Histogram of Inferior Fish Sauce Protein Content

Sensory Quality

Based on sensory test sauce from the raw material of hydrolyzate inferior fish with addition various kind and procentage of thickening agent applied on the fried rice processed product with simplified seasoning comprises color, scent, flavor, thickness and the overall, more information will be outlined and discussed as follows,

Preferred Color

Hedonic test result of sauce color from the raw material of hydrolyzate inferior fish with addition various kinds and procentage of thickening agent produced can be seen in Figure 7.

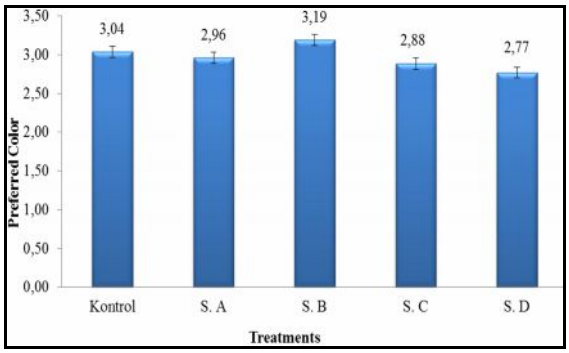

Figure 7. Histogram of Inferior Fish Sauce Preferred Color

Preferred Scent

Hedonic test result of sauce scent from the raw material of hydrolyzate inferior fish with addition various kinds and procentage of thickening agent produced can be seen in Figure 8.

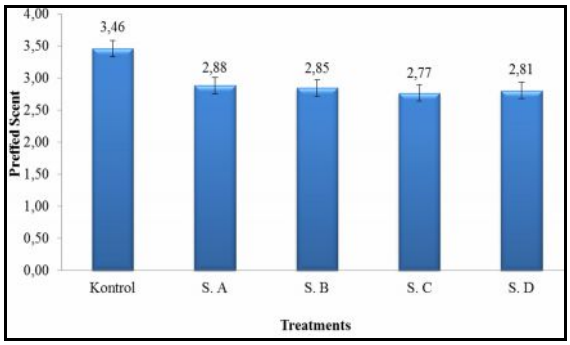

Figure 8. Histogram of Inferior Fish Sauce Preferred Scent

Preferred Flavor

Hedonic test result of sauce flavor from the raw material of hydrolyzate inferior fish with addition various kinds and procentage of thickening agent produced can be seen in Figure 9.

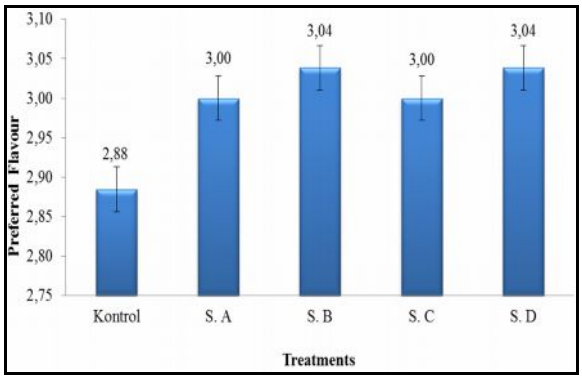

Figure 9. Histogram of Inferior Fish Sauce Preferred Flavor

Preferred Viscosity

Hedonic test result of sauce viscosity from the raw material of hydrolyzate inferior fish with addition various kinds and procentage of thickening agent produced can be seen in Figure 10.

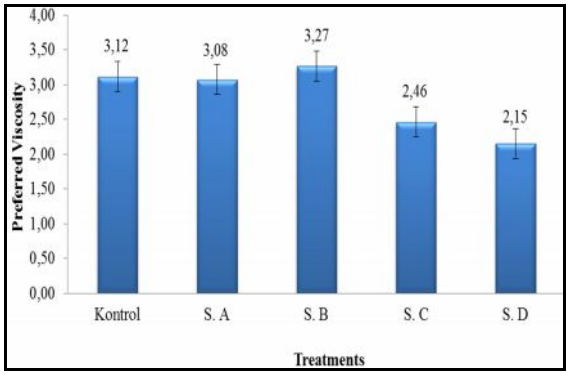

Figure 10. Histogram of Inferior Fish Sauce Preferred Viscosity

Overall

Hedonic test result of sauce overall from the raw material of hydrolyzate inferior fish with addition various kinds and procentage of thickening agent produced can be seen in Figure 11.

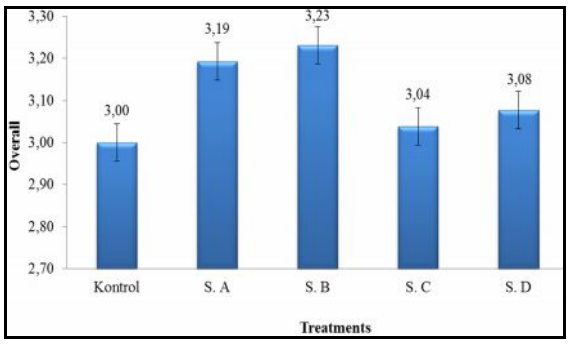

Figure 11. Histogram of Inferior Fish Sauce Overall

Effectiveness Test

Observation result of effectiveness test mainly used to determining which treatment with the highest or the best value for every parameter of inferior fish sauce observed. Histogram of effectiveness test can be seen in Figure 12.

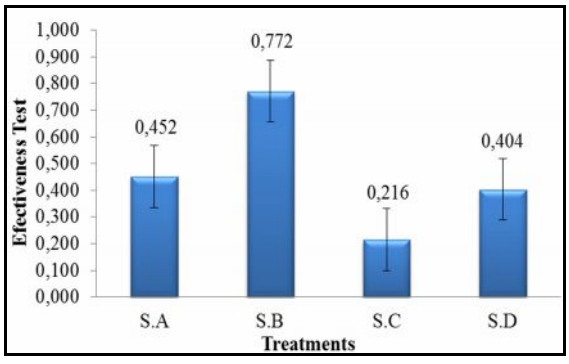

Figure 12. Histogram of Inferior Fish Sauce Effectiveness Test

Discussion

Viscosity

Figure 1 shows that S.B, treatment contains the highest viscosity value of 9,79 cp if compared with S.A; S.C; and S.D treatments. This is due to hydroxil group or DS (*degree of substitution*) on the CMC provide a change in the viscosity properties. CMC has DS value of 0,60 – 0.95 and molecular weight between 17.000 – 300.000 g/mol with DP 80 – 1500, so has the good nature as thickening agent, whilst the thickening agent of maltodextrin has molecular weight $\pm$ 1800 g/mol with DE 10[8]. The thickening agent of CMC has the very strong ability to absorbs water if compared with maltodextrin. CMC grains which are hydrophilic absorbs the water and swell up, so the water could not moved freely and the solution becomes more stable indicated with its viscosity increased[9].

Maillard Product

Figure 2 shows that S.B treatment contains the highest Maillard product of 0,43 AU if compared with S.A; S.C; and S.D treatments. That is suspected the thickening agent of CMC 1% has the higher ability to water binding than maltodextrin so the water in fish sauce would be reduced due to the more intensive of manufacturing brownish red color process (melanoidin), besides CMC has granular particles which could retin flavor and brownish red color (melanoidin) which formed on the inferior fish sauce[9]. Maillard reaction is reaction between carbonil group and amina primer group. This amina group obtained from the result of natural protein breakdown on the material[10].

Color

Figure 3 shows that S.B treatment contains the highest brightness (L) of 44,38 if compared with S.A; S.C; and S.D treatments. Type of added thickening agent can increasing the brightness level of the hydrolyzate base sauce of inferior fish. Stated that CMC particles can enveloping the color of brownish red produced by the material so the color of sauce produced will be brighter[11]. This is related to Maillard product that is reaction which allows the formation of flavor and brown color. The higher concentration of added CMC thickening hence the higher level of brightness of the produced inferior fish sauce, and vice versa the lower concentration of added CMC thickening and without the addition of thickening agent so the brightness level of inferior fish sauce will also reduced (dark).

Soluble Protein

Figure 4 shows that S.D. treatment contains the highest soluble protein value of 8,425 mg/ml if compared with S.A; S.B; and S.C treatments. That addition of various kinds and procentage of the thickening agents which added is indicated increasing total solid on the inferior fish sauce which lead to its solubility decreasing or low[12].

The addition of thickening agent both CMC and maltodextrin with 1% procentage will lead to its low solubility. The existence of CMC or maltodextrin in the solution tend to form crosslinking in the solvent molecule will trapped inside it so it occured to mobilization of solvent molecule which formed the rigid molecular structure, so it lead to low solubility [13].

Ash Content

Figure 5 shows that S.D. treatment contains the highest ash content value of 2,68 if compared with S.A; S.B; and S.C treatments. It is due to S.D treatment without addition of the thickening agents, so S.D treatment only wet hydrolyzate of inferior fish. That statement stated that mineral contents of fish is very high so it could increase ash content on the inferior fish sauce. Ash content is related with a mineral material[14]. Ash is organic substance of organic waste material of waste combustion of product of an organic agent[15].

Protein Content

Figure 5 shows that S.D treatment contains the highest protein content (7,52%) if compared with S.A; S.B; and SC treatments. It is due to the addition of type of thickening agent wich will decrease protein content on the inferior fish sauce. The addition of thickening agent both CMC or maltodextrin will lead to decreasing of protein content on that fish sauce. That the addition of type of thickening agent both maltodextrin and CMC at the same unit weight will decreasing volume or weight of wet hydrolyzate of protein content which will decrease the protein content on that inferior sauce[12].

Sensory Quality

Figure 7 on the preferred color shows that fish sauce treatment with addition of various kinds and procentage of different thickening agents but with equal consistency namely S.A; S.B; S.C; and S.D whilst compared with sauce treatment which available in the market (control), the highest preferred color value on the S.B treatment of 3.9 (rather like). It is due to CMC could envelop brownish red color generated by the agent[11]. Based on the color parameter, S.B tratement has the brighter sauce color and the panelists prefer fish sauce with the brighter color.

Figure 8 on the preferred scent shows that fish sauce treatment with addition of various kinds and procentage of different thickening agents but with equal consistency namely S.A; S.B; S.C; and S.D tend to subject on the preferred scent value if compared with control treatment. Inferior fish sauce on the addition of thickening agents treatment with various kinds and procentage of thickening agents on average contain preffered value that is not much defferent ranged between 2,77 – 2,88 (unlike). It is due to S.A; S.B; S.C; and S.D treatments only using wet hydrolyzate of inferior fish so its stench remain too strong. This stench due to protein decomposition[13]. The highest preferred scent value of fish sauce on the control (sauce in the market) of 3,46 (rather like). It is due to control has savory scent because on the manufacturing of market sauce (control) added with flavor enhancer (mononatrium glutamat, dinatrium 5 inosinat and dinatrium 5 guanilat) which lead to savory scent and preferred by the panelists.

Figure 9 on the preferred flavor shows that fish sauce treatment with addition of various kinds and procentage of different thickening agents but with equal consistency namely S.A; S.B; S.C; and S.D whilst compared with sauce treatment in the market, the lowest preferred flavor value of inferior fish sauce is the market sauce (control) of 2,88 (unlike). It is due to its manufacturing using flavor enhancer agents (mononatrium glutamat, dinatrium 5 inosinat and dinatrium 5 guanilat) so its savory flavor generated too strong and leave slightly bitter taste in throat, so on the control treatment of its preferred flavor value is less preferred by the panelists. The flavor of inferior fish sauce with addition of different thickening agents treatment and equal concentration on average have preffered value that is not much different ranged between 3,00 – 3,04 (rather like). It is due to inferior fish contain high and natural savory flavor (*umami*). Hydrolyzate of inferior fish can be used as raw material of seasoning which will be applied to the food[16].

Figure 10 on the preferred viscosity shows that fish sauce treatment with addition of various kinds and procentage of different thickening agents but with equal consistency namely S.A; S.B; S.C; and S.D whilst compared with sauce treatment in the market, the highest preferred viscosity value of inferior fish sauce is S.B treatment of 3,27 (rather like). It is due to inferior fish sauce with addition of thickening agent of 1% CMC can increase viscosity of inferior fish sauce. CMC as thickening agent has a very strong water absorption ability. The hydrophilic nature of CMC grains is absorbs the water and swell up, so the water could not move freely and the solution becomes more stable indicated with increased viscosity[9]. The lowest preferred viscosity value of inferior fish sauce is S.D treatment of 2,15 (unlike). It is due to sauce without addition of thickening agent is not using thickening agent due to only using wet hydrolyzate of inferior fish, so the sauce produced is not thick. According viscosity parameter (thickening), S.B treatment contain the thicker viscosity and the panelists preferred fish sauce contain high viscosity.

Figure 11 on the preferred overall shows that fish sauce treatment with addition of various kinds and procentage of different thickening agents but with equal consistency namely S.A; S.B; S.C; and S.D whilst compared with sauce treatment in the market, the highest preferred overall value of inferior fish sauce is the addition of different thickening agent treatment with 1% concentration on average contain the preferred overall value that is not much different ranged between 3,04 – 3,23. The lowest preferred overall value of inferior fish sauce is the market sauce (control) of 3,00 (rather like). It shows that the preferred sauce by the panelist overall for color, scent, flavor and viscosity is S.B treatment (3,23) (rather like until like).

Effectiveness Test

Figure 12 shows that the value of effectiveness test on the fish sauce with addition of various kinds and procentage of thickening agent contain the highest value is S.B treatment of 0,722. The thickening agent 1% CMC is more appropriately used as thickening agent, it is due to CMC has hydrophilic nature and its stability can be maintained[9]. On the fish sauce with addition of various kinds and procentage of thickening agent which contain the lowest valur is S.C treatment of 0,216.

Conclusion

1. Characteristic of fish sauce produced from addition of type of thickening agent 1% CMC has good sauce properties if compared with addition of thickening agent 1% maltodextrin, 0,5% maltodextrin + 0,5% CMC or without addition of thickening agent. Addition of thickening agent 1% CMC on the inferior fish sauce thicker viscosity value of (9,79 Pa.S), higher Maillard product of (0,43 AU), brighter brightness level of (44,38). While without addition of thickening agent both 1% maltodextrin and CMC producing fish sauce with soluble protein content of (8,425 mg/ml), ash content of (2,68%), and high protein content of (7,52%).
2. Addition of 1% CMC is the most appropriate treatment according to the result of effectiveness test with the highest value of (0,772). The produced sauce is preferred by the panelist overall for the parameter of color, scent, flavor and viscosity with value ranges (3,23) (rather like).

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