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PHYSICOCHEMICAL CHANGES AND SENSORY QUALITY OF LIQUID SMOKED MILKFISH NUGGETS

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ABSTRACT

Liquid smoke method is an alternative to traditional smoking techniques, which aims to reduce polycyclic aromatic hydrocarbon (PAH) levels in the final product by degrading cellulose, hemicellulose, and lignin during pyrolysis of wood using a high-temperature combustion process. Research in liquid smoke is readily available in its application on raw fish product. However, less information is available in the ready-to-serve-product made of fish. Therefore, this study aimed to determine the effects of varying types and concentrations of liquid smoke on the sensory and proximate characteristics of milkfish nuggets, depending on the hardness, deformation, gel strength, organoleptic, and proximate testing. Parametric data were utilized for variance analysis and Tukey's test. Non-parametric data, such as organoleptic and hedonic data, were analyzed using the Kruskall–Wallis test and the Mann–Whitney test. Varying types and concentrations of liquid smoke were found to significantly affect the hardness, deformation, gel strength, and protein and carbohydrate levels, as well as the organoleptic and hedonic properties on a 9-point scale starting with 1 to 9, with 1 as very unpleasant and 9 very pleasant ($p < 0.05$), but had no significant effect on the moisture or fat content of the resulting milkfish nuggets ($p > 0.05$). The best treatment was found to be 3 % re-distillation liquid smoke. Overall, the organoleptic and hedonic properties of milkfish nuggets processed with filtered and double-distilled liquid smoke were acceptable to be applied. Moreover, determining the type and concentration of liquid smoke is important consideration in the production of milkfish nugget in order to produce best physical and chemical qualities. Furthermore, milkfish nuggets were found to contain essential amino acids and fatty acids, which can be obtained when 3 % re-distilled liquid smoke was utilized to produce milkfish nuggets. This finding practically reveals the method and composition of milkfish nugget liquid, which was more likely to have the highest preference for consumers and may open the opportunity for the food industry to develop more derivative products from fish.

Key words: Liquid smoke, Milkfish nuggets, Texture, DHA, EPA, Proximate

INTRODUCTION

Smoking has been a traditional method to preserve fresh fish [1]. In Semarang Indonesia, being a coastal region, smoking has been used as a primary food processing method as it adds some unique flavor and color to their products, which can be attributed to compounds such as formaldehyde, carboxylic acids, and phenols [2]. As a national industry, the production of smoked fish in Central Java Province has reached 5 tons per day from 0.5 million tons [3, 4]; this is proof that the government highly supports the smoked fish producers [5]. There are several smoking methods in use in Indonesia, and these include a variety of applied wood such as "kesambi wood," which is known to produce traditional "sei" smoked beef [6]. Smoking methods like the kiln model are also utilized to enhance the flavor of the food, not compromising its healthy features [7].

Polycyclic aromatic hydrocarbons (PAHs) mainly exist in smoke, which is the largest class of chemical compounds, containing two or more fused aromatic rings made up of carbon and hydrogen atoms, and they are often categorized as genotoxic agents [8]. Polycyclic aromatic hydrocarbons are formed during the incomplete combustion processes that may occur whenever wood, coal, or oil are burned [8], most consumers are aware of their toxicity and carcinogenic activity [7].

Liquid smoke method may be an opportunity to reduce PAH levels in the final product since the degradation of cellulose, hemicellulose, and lignin occurs during pyrolysis of wood using a high-temperature combustion process [9]. Liquid smoke is applied in various foods like fruits and vegetables to produce a unique taste and to lengthen their shelflife due to flavor enhancement and antibacterial properties [10].

There is still limited literature on the study of liquid smoke in derivative products, such as fish nuggets. While it can also be combined with other ingredients including local leaves [12], with its edible waste fish product re-utilized [13], fish nuggets can be varied, which may enhance consumer preference to fish product [11]. Furthermore, fish products may help in curbing the demand for food with high nutritional value [14]. This study has analyzed how liquid smoke method can affect the quality of fish nugget.

MATERIALS AND METHODS

Materials

Milkfish (weight 250 ± 25 g) were obtained from a fish culture in the northern part of Semarang city. With help from the Marine Science Techno Park, Diponegoro University, liquid smoke method was utilized from coconut shells. The raw material of liquid smoke was coconut shell. The manufacture of liquid smoke was done according to the previous method using the pyrolysis method with temperature of 450 ˚C [7]. The production of liquid smoke was conducted in manufacturing nuggets, flour, breadcrumbs, pepper, and onions were purchased and used. Liquid smoke was added during dough making along with flour, breadcrumbs, pepper, and onion. For proximate analysis, H_2SO_4, HCl, NaOH, HCl, $HBrO_3$, and N-hexane were used. Stainless steel knife, food processor, and steamer from a local manufacturer were utilized for the

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production of fish nuggets. Texture analysis was conducted in the Fisheries and Marine Sciences Faculty, Diponegoro University. Proximate analysis was conducted using an oven, Kjeldahl apparatus, Soxhlet apparatus, and furnace coming from the Central Laboratory for Research and Services, Diponegoro University.

Analysis of total phenol content in liquid smoke

The total phenol content of the liquid smoke was measured using the Folin–Ciocalteu method as described by Amin *et al*. [15], with slight modifications using as gallic acid equivalent at a range 0.01%–0.05 %. Estimation of total carbonyl content was done according to JECFA [16].

Analysis of PAH

Homogenized samples were hydrolyzed with a solution of potassium hydroxide in ethanol and extracted with cyclohexane. The cyclohexane solution was washed with water and with a mix of methanol and water, then re-extracted with an N, N-dimethylformamide/water (9:1) blend, and then repeatedly extracted with cyclohexane. After cyclohexane solution purification on Silica SPE column, the sample was concentrated and analyzed on an Agilent Model 6890 gas chromatograph equipped with the mass selective detector Model 5973 [9].

Preparation of milkfish nuggets

The filleted milkfish were ground into a pulverized meat, seasoned with pepper, onion, salt, and pulverized refined sugar. The dough was prepared by mixing minced fish, spices, flour, and liquid smoke at concentrations of 1 %, 3 %, and 5 %. The dough was steamed for 30 min and then cooled for 20 min before getting smeared with butter and in a breadcrumb mixture. The nuggets were stored at 4 °C for analysis the next day. The liquid smoke was tested for total phenol, total carbonyl, total radical scavenging activity, and PAH components. The milkfish nuggets were tested for hardness, deformation, and gel strength, as well as sensory evaluation, which is an organoleptic and hedonic test. Proximate analysis which contains water, protein, fat, ash, and carbohydrate contents, was also performed.

Textural analysis

The textural analysis consisted of hardness, deformation, and gel strength testing and was performed for nugget banding using a texture analyzer (Model TA Plus, Lloyd Instruments, London, UK). Samples of milkfish nuggets with the size of 2.5 x 2x0.5 cm each were prepared for textural analysis. A 5 mm probe ball was used, and the texture analyzer was set to 60 mm/min. Gel strength (gf.cm) was calculated by the multiplying violence (gf) and deformation (cm). Texture analysis was conducted in the Fisheries and Marine Sciences Faculty, Diponegoro University. Proximate analysis was conducted using an oven, Kjeldahl apparatus, Soxhlet apparatus, and furnace coming from the Central Laboratory for Research and Services, Diponegoro University.

Sensory analysis

A list of 30 trained staff as the panelists assessed the organoleptic and hedonic properties of the milkfish nuggets. Organoleptic testing was performed according to the National Standardization Agency of Indonesia No: SNI-01-6683-2002 [17], while hedonic testing was conducted according to the National Standardization Agency of Indonesia Number: SNI 01-2346-2006 [18]. The hedonic test methods were based on BSN [18] according to SNI 01-2346-2006 and sensory testing in fishery guidelines. The sensory attributes that were examined were appearance, smell, taste, and texture on a 9-point scale starting with 1 to 9, with 1 as very unpleasant and 9, very pleasant.

Analysis Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA)
Analysis of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) were done using gas chromatography [method B]. Briefly, lipid was initially extracted from the homogenate of nugget with chloroform and methanol mixture and then converted to fatty acid methyl esters using 5% hydrochloric methanol.

Proximate analysis
The proximate analysis was done following the AOAC methods using analytical Nos. 928.08, 950.46, 920.153, and 960.39 for moisture, protein, fat, and ash contents, respectively [19].

Statistical analysis
All treatments were carried out in triplicates, and data were processed using analysis of variance (ANOVA). Group differences were identified using Tukey's test. Some non-parametric data were analyzed with the Kruskall–Wallis test followed by the Mann–Whitney test using the SPSS software (SPSS 17.0 for Windows; SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Liquid smoke characteristics
With reference to previous research, this study used distilled, filtrated, and re-distillated liquid smoke [20]. Table 1 explains the decrease the total phenol contents, carbonyl contents, and radical scavenging activity. Distillation and filtration of liquid smoke might reduce the total phenol content from 0.0555 ± 0.0011 to 0.0364 ± 0.0021 that was indicating the loss of 34.41 %, while re-distillation decreased phenol content from 0.0555 ± 0.0011 to 0.0164 ± 0.0020, indicating the loss of 70.45%. This may be explained by the loss during the heating process. The phenol and carbonyl contents and the radical scavenging activity of the nano encapsulated liquid smoke were the highest among the liquid smoke processing types examined.

However, the total phenol content of the liquid smoke in this study was lower than that reported by Lombok *et al*. [21], who showed that total phenol content was around 2.25 % when liquid smoke is distilled at a temperature between 100 and 120 °C. Meanwhile, Nithin *et al*. [2] reported that phenol content of liquid smoke derived from coconut shells was only 0.01348 %, slightly lower than the findings in this research. The remarkable variation of phenol content in liquid smoke may be attributed to the applied raw material and distillation temperature [22], which can affect the liquid smoke types. While the liquid smoke with a low phenol content in this study might serve as a flavorant only, rather than as antibacterial and antioxidant enhancer [22], Budaraga *et* *al.* [10] reported that phenol and carbonyl contents increased in distilled and filtered liquid smoke, providing specific flavor and color to the fish meat.

Based on gas chromatography analysis, six PAH compounds were detected: naphthalene; acenaphthene; phenanthrene; pyrene; benzo-a-antrazene; and benzo-a-pyrene (Table 2). Four compounds were detected in the distilled liquid smoke, five in the filtered smoke, and three compounds in the double-distilled liquid smoke. Pyrene content was highest in filtered liquid smoke and lowest in the re-distilled type; benzo-a-antrazene and benzo-a-pyrene contents were highest in the re-distilled type. As stated by Ledesma *et al.* [23], PAH are carcinogenic and mutagenic compounds that should be found in lower amounts in food. This was linear with the statement from World Health Organization, the International Agency for Research on Cancer, the European Food Safety Authority, and the US Environmental Protection Agency that PAH in smoked meat should be no more than 2 µg/kg as regulated by European Regulation No. 835/2011 [23]. Thus, double-distilled liquid smoke is a safer method of producing fewer carcinogenic and mutagenic compounds.

Texture

The hardness of milkfish nuggets is dependent on the different types and concentrations of liquid smoke (Fig. 1). ANOVA showed an interaction between the type and concentration of liquid smoke ($p < 0.05$). For example, 3% double-distilled liquid smoke produced the highest hardness. Deformation data are presented in Fig. 2 which indicates that significant interaction between smoke type and concentration can deform the milkfish nuggets. Deformation tended to increase in nuggets with a 3 % liquid smoke concentration but decreased at 5 % concentration. The 3 % filtered smoke treatment had a higher deformation value than other treatments, but this showed no significant difference at 1 % concentration of the re-distilled smoke.

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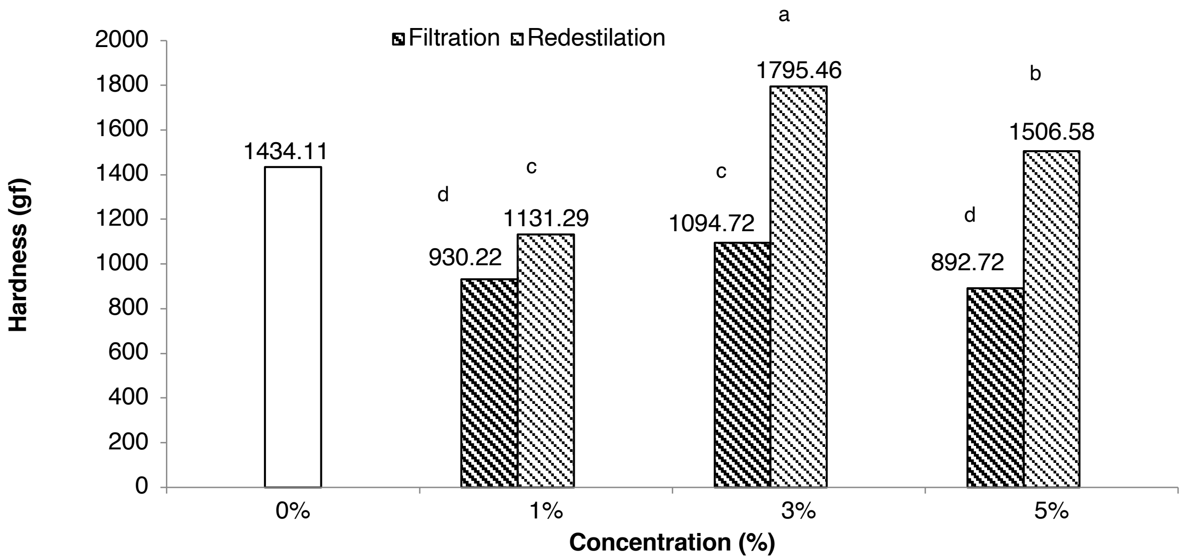

Figure 1: Hardness of milkfish nuggets containing different types and concentrations of liquid smoke

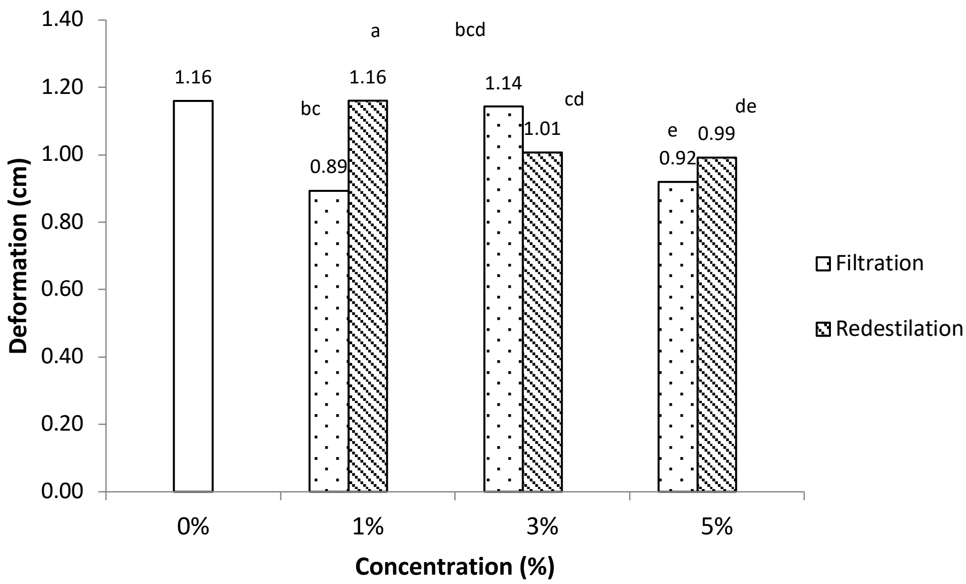

Figure 2: Deformation of milkfish nuggets with different types and concentrations of liquid smoke

There was some interaction between the type and concentration of liquid smoke on the resulting nuggets' gel strength ($p < 0.05$). Gel strength banding is presented in Fig. 3.

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The filtered and re-distilled liquid smoke produced nuggets with similar gel strengths. Milkfish nuggets treated with re-distilled liquid smoke had higher gel strengths than those treated with filtered liquid smoke, with the gel strength increasing in milkfish nuggets with 3% liquid smoke concentration but decreasing with 5 % liquid smoke concentration.

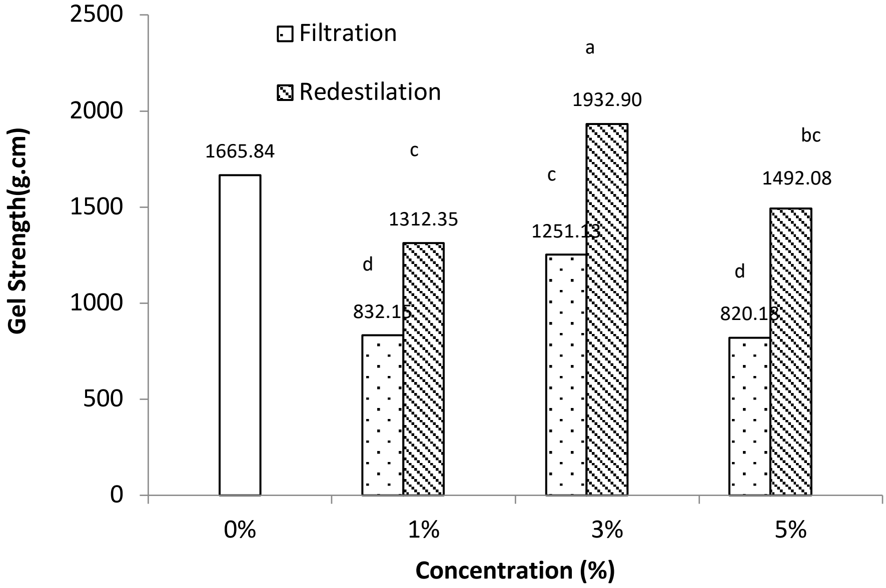

Figure 3: Gel strength of milkfish nuggets with different types and concentrations of liquid smoke

Overall, the type and concentration of liquid smoke produced different effects on texture parameters (hardness, deformation, and gel strength). The use of liquid smoke can increase the hardness and gel strength but decrease deformation. Meanwhile, the decreased nugget deformation in all trials could be due to breadcrumbs coating the nugget surface since breadcrumbs have a relatively low plastic deformation, so including them in the nugget results in lower deformation, where the decreased deformation can partially be explained by the higher phenol content of liquid smoke, which cross-links nuggets' protein molecules to enhance the hardness and gel strength [24].

Sensory analysis

The type and concentration of liquid smoke can significantly affect the organoleptic value of the milkfish nuggets (Table 3). Based on the minimum level for acceptable organoleptic score (score > 7), filtered or re-distilled liquid smoke milkfish nuggets were suitable for consumption. The brown color of nuggets was due to carbonyl compounds reacting with the proteins in a Maillard reaction [25]. Thus, appearance generally decreased with increasing concentration of added liquid smoke, as the nuggets became darker. As a consequence, the panelists provided much lower score.

The highest appearance score was recorded in milkfish nuggets with 1% filtered liquid smoke, similar to the milkfish nuggets without liquid smoke (control).

An increase by one per cent in the concentration of re-distilled liquid smoke contributed to the increase in the appearance score. This may be explained due to the reduction of the score of smell from 8.03±1.05 to 7.79±0.85. However, 5% re-distilled liquid smoke reduced the typical smell of fish but increased the smell score of smoke, resulting in low score values. Amazingly, 5 % re-distilled liquid smoke received the highest taste score, although they were not significantly different from those of 3 % re-distilled liquid smoke. Smoke flavor is generally recognized as safe and is considered by both USDA and FDA as a natural flavoring [26], with the lowest specific sense value being obtained at 5 % liquid smoke concentration, because of the strong flavor from smoke. Liquid smoke can be applied as a dip or a drench to color and flavor cooked ready-to-eat meats [27] and added directly to other food products such as barbecue sauce, dry crispy snacks, canned baked beans, and canned fish and shellfish [28]. In metropolitan areas where the discharge of smoke to the environment is restricted, liquid smoke may be substituted [25].

Hedonic analysis

Overall, milkfish nuggets were preferred with liquid smoke rather than the one without. The average value for nuggets containing 5 % liquid smoke was > 7, which is favorable to consumers.

All milkfish nuggets containing filtered or re-distilled liquid smoke were highly rated on their appearance by the panelists; and nuggets with 1% or 3% re-distilled liquid smoke received an average value of 8. The appearance of nuggets with 1 % filtered liquid smoke was higher than that of 3% and 5%, but lower than those of 3 % of re-distilled liquid smoke. Milkfish nuggets with 3 % or 5% filtered or nano encapsulated liquid smoke, and 1% re-distilled liquid smoke were highly preferred by the panelists.

Adding liquid smoke also brings significant effect to the taste of the milkfish nuggets as earlier reported by Widiastuti *et al.* [29]. Three per cent re-distilled liquid smoke provided the highest flavor value, while the lowest flavor value was achieved by 3 % filtered liquid smoke. While texture of the nuggets containing 3 % re-distilled liquid smoke was the most preferred, as this texture preference was observed, nuggets with 5 % filtered liquid smoke provided higher score than those of 1 % and 3 % (Table 4). Though subjective preferences play a large role for most consumers, these findings prove empirically that the liquid smoke flavor of milkfish nuggets was preferred by consumers. This finding is in line with Martinez and Machado [30], which confirmed that the majority of the consumers indeed liked liquid smoke flavoring.

Proximate analysis

Although liquid smoke concentration does not affect water content, the distilled and filtered liquid smoke provided higher value than the double-distilled. The water content of all milkfish nuggets exceeded the Indonesian National Standards of 60 %. Values in this research were higher than those reported by Chen *et al.* [31], who found that nugget water content varied from 31.60 % to 45.27 % in the final product due to further frying and heating. However, Suprayitno *et al.* [11] reported that nuggets combined with tofu had a water content of around 61.16 %–65.45 %.

The type and concentration of liquid smoke affected the protein content of milkfish nuggets ($p < 0.05$), with the value ranging from 13.56 % to 15.62 % (Table 5). The protein content of milkfish nuggets in this research met the Indonesian National Standards, which is > 12 %. This was found to be similar to the findings of Lima *et al.* [32], which reported the protein content of Nile tilapia nuggets to be between 12.85 % and 16.12 %. Jayasinghe *et al.* [33] and Sarkar *et al.* [34] have found higher protein contents in fish nuggets, which were around 14.72%–20.28 % and 15.5%–19.3%, respectively, attributed to addition of high-protein flour and vegetables. Fat content of milkfish nuggets was 3.17 %–3.75 %, which is within the Indonesian National Standards (maximum of 20 %) but lower than that reported by Jayasinghe *et al*. [33] and Sarkar *et al.* [34], where liquid smoke concentration significantly affected the fat content of milkfish nuggets, which increased along with the increase in concentration.

Amino acid analysis
Milkfish nuggets in this study contain essential and non-essential amino acid. Highest amounts of non-essential amino acid were found in glutamic acid at a range of 14353.08–19431.07 mg/kg (Table 6) as well as in lysine (essential amino acid) at a range of 7577.63–10,385.90 mg/kg. These results were higher than those earlier reported by Liputo *et al.* [35] for large snout goby (*Awaous melanocephalus*) fish nuggets, which contained 2410 and 2110 mg/kg glutamic acid and lysine, respectively. This is likely because milkfish have higher essential amino acid contents than large snout gobies.

Determination of EPA and DHA
Borlongan and Benitez [36] reported that milkfish grown in seawater and freshwater contain different levels of fatty acids. Milkfish grown in seawater contained higher polyunsaturated fatty acid (PUFA), specifically omega-3 fatty acids [37]. Wan-Rosli *et al.* [38] also reported that saltwater-raised fish contained higher levels of long-chain omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) than those raised in freshwater. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are essential nutrients that are fundamental to brain function and enhance quality of life and lower the risk of premature death [39]. Because of the high levels of EPA and DHA, as shown in Table 7, the use of milkfish as a raw material for nuggets offers health benefits. The highest EPA and DHA levels were found in milkfish nuggets with 1 % filtered liquid smoke. As shown in Table 7, increasing the concentration of filtered liquid smoke lowered the EPA and DHA levels, whereas increasing the concentration of re-distilled liquid smoke increased their levels. These data correlated with total phenol data (Table 1) show that distilled and filtered liquid smoke had higher total phenol than doubled distilled liquid smoke. Sufficient amount of phenol is needed for antioxidant activity of liquid smoke that prevents the fatty acid content. Phenol levels that are safe for consumption are between 0.02% - 0.1% or 200 ppm–1000 ppm.

In what may likely have been due to the lower lipid contents of milkfish compared with the large snout goby (*Awaous melanocephalus*), reported at 14.43%, the levels of EPA found were lower than those of large snout goby fish nuggets (*Awaous melanocephalus*), as reported by Liputo *et al.* [30]: 20.1–37.3 mg/100 g compared with 3130 mg/100 g.

CONCLUSION

Varying the types and concentrations of liquid smoke were found to significantly affect the hardness, deformation, gel strength, protein, and carbohydrate levels, as well as the organoleptic and hedonic properties, but had no significant effect on the moisture or fat content of the resulting milkfish nuggets. Based on the observed parameters, the best treatment was 3 % re-distillation liquid smoke. This finding practically reveals the method and composition of milkfish nugget liquid, which is more likely to have the highest preference for consumers. The use of 3% liquid smoke was recommended to be applied to the production process of nugget. On the other hand, dissemination & education to society should be done continuously in collaboration with the government.

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Table 1: Characteristics of liquid smoke obtained using distillation, distillation and filtration, and double distillation processes include superscript for the reader to know any significant differences that may exist in all tables

Parameter	Type of Liquid smoke		
	Distilled Only	Distilled and Filtered	Double distilled
Total phenol (%)	0.0555 ± 0.0011[a]	0.0364 ± 0.0021[b]	0.0164 ± 0.0020[c]
Total carbonyl (%)	0.4986 ± 0.0018[a]	0.4550 ± 0.0035[a]	0.3776 ± 0.0016[a]
Total Radical Scavenging Activity (RSA) (%)	85.5308 ± 0.1211[a]	71.8322 ± 0.121[b]	22.5171 ± 0.1211[c]

Different letters at the same line indicate significant differences between treatments (P<0.05)

Table 2: Polycyclic aromatic hydrocarbon (PAH) contents in distilled, distilled and filtered, and double-distilled liquid smoke

Parameter	Type of Liquid smoke		
	Distilled only	Distilled and filtered	Double distilled
Naphthalene (ppm)	nd	nd	nd
Acenapthane (ppm)	nd	1.32	nd
Phenantrene (ppm)	0.55	0.44	nd
Pyrene (ppm)	2.07	8.34	2.87
Benzo@Antrazene (ppm)	2.93	2.43	16.55
Benzo@Pyrene (ppm)	29.98	8.26	105.79

Table 3: Organoleptic values of milkfish nuggets with different types and concentrations of liquid smoke

Liquid smoke type	Concentration	Specification				
		Appearance	Smell	Taste	Texture	Total
Control	0%	8.00 ± 0.89[a]	8.18 ± 0.87[a]	8.31 ± 0.84[a]	8.43 ± 0.9[a]	8.23 ± 0.5[a]
Distilled and filtered	1%	8.09 ± 0.93[a]	7.93 ± 0.86[a]	7.73 ± 0.83[a]	7.07 ± 0.83	7.71 ± 0.41[b]
	3%	7.39 ± 0.87[b]	7.54 ± 1.00[b]	7.07 ± 0.93[b]	7.1 ± 0.95[b]	7.29 ± 0.62[c]
	5%	7.27 ± 0.83[b]	7.42 ± 0.86[b]	6.79 ± 1.10[c]	7.48 ± 1.17[b]	7.24 ± 0.45[c]
Double distilled	1%	7.8 ± 1.01[a]	8.03 ± 1.05[a]	8.08 ± 1.18[a]	7.13 ± 0.91[b]	7.76 ± 0.45[b]
	3%	7.12 ± 1.04[b]	7.26 ± 0.79[b]	7.37 ± 0.89[b]	7.83 ± 0.78[a]	7.39 ± 0.54[c]
	5%	7.87 ± 0.94[a]	7.79 ± 0.85[a]	8.09 ± 0.89[a]	8.46 ± 1.13[a]	8.05 ± 0.56[a]

Different letters at the same row indicate significant differences between treatments (P<0.05)

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Table 4: Hedonic scale of milkfish nuggets with varying type and concentration of liquid smoke

Liquid smoke type	Concentration	Appearance	Color	Smell	Taste	Texture	TOTAL
Control	0%	7.52 ± 0.67[a]	7.62 ± 0.88[a]	7.52 ± 0.82[a]	7.24 ± 0.71[b]	7.53 ± 0.5[a]	7.49 ± 0.39[b]
	1%	7.34 ± 0.52[a]	7.57 ± 0.65[a]	7.17 ± 0.55[b]	7.07 ± 0.83[b]	7.06 ± 0.69[b]	7.24 ± 0.37[b]
	3%	7.12 ± 0.67[b]	7.4 ± 0.56[b]	7.01 ± 0.79[b]	7.29 ± 0.74[b]	7.07 ± 0.68[b]	7.18 ± 0.38[b]
Distilled and filtered	5%	7.14 ± 0.55[b]	7.77 ± 0.91[a]	6.98 ± 0.79[b]	7.51 ± 0.67[a]	7.11 ± 0.55[b]	7.30 ± 0.42[b]
	1%	7.39 ± 0.77[b]	7.37 ± 1.18[b]	7.32 ± 1.00[b]	7.13 ± 0.78[b]	7.36 ± 0.57[a]	7.31 ± 0.57[b]
Double distilled	3%	7.58 ± 0.79[a]	7.86 ± 0.76[a]	7.6 ± 0.79[a]	7.95 ± 1.09[a]	7.52 ± 0.52[a]	7.70 ± 0.42[a]
	5%	7.26 ± 0.79[b]	7.36 ± 0.69[b]	7.39 ± 1.19[b]	7.1 ± 0.77[b]	7.14 ± 1.26[b]	7.25 ± 0.52[b]

Specification columns: Appearance, Color, Smell, Taste, Texture.

Different letters at the same row indicate significant differences between treatments (P<0.05)

Table 5: Proximate content of milkfish nuggets with different types and concentrations of liquid smoke

Liquid smoke type	Conc.	Water (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)
Control	0%	61.03 ± 0.38	13.563 ± 0.19	4.65 ± 0.17	2.57 ± 0.23	11.29 ± 1.42
Distilled and filtered	1%	64.49 ± 1,10[A]	14.77 ± 0,22[ab]	3.43 ± 0.09[Bp]	1.58 ± 0.13[Bp]	11.52 ± 1,90[d]
	3%	63.32 ± 0,55[A]	15.62 ± 0,2[a]	3.75 ± 0.07[Bpq]	1.29 ± 0.17[Bp]	12.86 ± 0,11[bcd]
	5%	63.40 ± 1,27[A]	13.75 ± 0,16[bc]	3.28 ± 0.1[Bq]	1.63 ± 0.22[Bq]	13.90 ± 0,88[bcd]
Double distilled	1%	60.62 ± 0,43[B]	13.77 ± 0,18[b]	3.17 ± 0.03[Bp]	1.69 ± 0.23[Bp]	17.11 ± 0,84[a]
	3%	60.62 ± 0,43[B]	15.48 ± 0.77[a]	3.68 ± 0.18[Bpq]	1.16 ± 0.12[Bp]	15.23 ± 2,07[bcd]
	5%	60.65 ± 2[B]	14.54 ± 0.32[ab]	3.72 ± 0.56[Bq]	2 ± 0.5[Bq]	14.75 ± 0,28[ab]

Parameter columns: Water (%), Protein (%), Fat (%), Ash (%), Carbohydrate (%).

Different capital letters (A, B) in the same column indicates significant differences in the type of liquid smoke (p< 0.05); different lowercase letters (p, q) in the same column indicates significant difference in concentration; different letters (a, b, c) in the same column indicates significant differences in the combination of the kind of liquid smoke and concentration

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Table 6: Amino acid concentrations of milkfish nuggets with different types and concentrations of liquid smoke

NO	Amino Acid (mg/kg)	Control	Distilled and filtered			Double distilled		
			1%	3%	5%	1%	3%	5%
1	Glysine	4396.12	4697.62	3497.21	4029.01	3663.04	3348.07	4828.60
2	L-Alanin	5267.70	5384.34	4275.04	4213.49	4365.61	4116.54	4747.38
3	L-Arginin	4656.05	5267.02	3633.20	4620.16	3936.76	3337.57	5236.54
4	L-Aspartad acid	8095.65	8428.30	6666.51	6297.52	6985.28	6438.94	6595.33
5	L-Glutamic acid	17236.49	19431.07	15187.63	14353.08	16167.59	16812.50	15817.15
6	L-Fenilalanin	3645.75	3897.67	2708.07	3614.36	3007.29	2776.10	4507.05
7	L-Histidin	3364.61	3343.61	2675.53	3210.67	2716.54	2512.84	3809.36
8	L-Isoleusin	3910.46	3904.30	3128.24	3190.11	3085.74	2997.54	4034.14
9	L-Leusin	7072.61	7615.43	5804.83	6266.61	5958.90	2997.54	7369.02
10	L-Lysine HCl	9869.78	10385.90	8241.22	7577.63	8606.18	7801.65	7960.78
11	L-Prolin	3858.03	4268.01	3346.93	3427.01	3530.16	3740.68	4224.14
12	L-Serin	3800.41	3701.79	2925.58	3159.27	2956.93	2923.89	4017.61
13	L-Threonin	4184.42	4101.84	3275.54	3159.27	3134.65	3149.98	4396.22
14	L-Tirosin	2896.62	2562.10	2148.71	2449.14	1981.70	2174.90	3195.77
15	L-Valin	4414.64	4410.33	3585.09	3604.73	3526.37	3430.33	4452.76

Table 7: EPA and DHA of milkfish nuggets with different types and concentrations of liquid smoke

Liquid smoke type	Concentration	Fatty Acid (mg/100 g)	
		EPA	DHA
Control	0%	29.85	44.55
Distilled and filtered	1%	37.3	53.1
	3%	20.1	28.3
	5%	26.5	38.9
Double distilled	1%	27.3	37.9
	3%	26.2	36.1
	5%	29.95	42.0

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