Effect of Heating Treatment of VCO By-product on Protein, Fat, Free Fatty Acid, Emulsification Capacity, and Fatty Acid Characteristics

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ABSTRACT

Virgin coconut oil (VCO) waste or blondo was a by-product, which still contains many important nutrients, and has a potency to be included for the production of nutritious food products. Blondo still contains a high moisture, and so to extend its shelf life and expand its application, it needs to be dried and changed in the powder form. This work was aimed to study the effect of heating time in the preparation of blondo powder on the physicochemical and fatty acid properties. VCO was prepared with a method of oil addition, and the blondo as a by-product was separated, dried with three different length of heat treatments (H1: 3 hours, H2: 6 hours, and H3: 12 hours with air oven drying), and grinded. The blondo powder was then evaluated for the content of protein, fat, and free fatty acid, emulsification capacity, and fatty acid characteristics. The results obtained indicated that a longer heating time resulted in a lower protein content, fat content, and emulsification capacity, but with a higher free fatty acid content. The fatty acid components contained in blondo powder which were characterized using GC-MS produced nine fatty acid components. They were lauric acid, myristic acid, heptadecanoic acid, linoleic acid, octanoic acid, decanoic acid, cis-11-eicosenoic acid, hexanoic acid and butyric acid; with the highest component was lauric acid ($C_{13}H_{26}O_2$). The blondo powder produced with those treatments was most likely to be incorporated in a food formulation.

Keywords: *Emulsification capacity, fatty acid characteristics, heat treatment, VCO by-product.*

1. INTRODUCTION

Blondo is a by-product of the manufacture of Virgin Coconut Oil (VCO) or the manufacture of pure coconut oil with a wet process, namely the process of extracting coconut oil from coconut milk [1]. Several studies also revealed that blondo might be utilized as antibacterial products [2], hand sanitizer [3], and even as flavour enhancer [4]. A high nutritional content of blondo has suggested that blondo may be used in the food formulation such as processed cheese, meat, cookies, and other processed foods. According to Wijana et al. [4], the blondo prepared from the manufacture of VCO contained protein of 16.38%, fat of 26.59%, and water of 30.32%. The nutritional content contained in blondo is quite high, especially the protein content. Therefore, blondo might be used as a basic ingredient for making a high protein food product. In addition to high protein content, blondo also has a fairly high fat content with the presence of vegetable oils that are good for health to facilitate metabolism in the body and become an additional source of energy [5]. However, the blondo is easily turn into spoilage, if it is not processed further within a few days. It will result in emitting an unpleasant odour, very pungent, and it may pollute the environment [6]. Therefore, the resulting blondo must be immediately dried and made in the form of flour. This study was aimed to examine the effect of drying time on the content of protein, fat, free fatty acids, and fatty acid characteristics.

2. MATERIALS AND METHOD

2.1. Materials

The material used was a mature coconut of 10-11 months old, characterized by dark brown skin. The samples were obtained from local farmers in Lambangi, South Konawe, Southeast Sulawesi.

The main chemicals used for the analysis, such as n-hexane, 96% alcohol, NaOH, and H_2SO_4 were of analytical grade.

2.2. Preparation of Blondo

The preparation of blondo began with the process of extracting coconut milk from a grated coconut, and dissolved in water with a ratio of 1:1. The resulting coconut milk was then incubated at room temperature for 3-5 hours to form two layers; water in the bottom layer, and cream in the upper layer. Then, the cream was separated, and added with Virgin Coconut Oil (VCO) with a ratio of 3:1. The mixture was then incubated for 24 hours, to form three layers, water in the bottom, blondo in the middle, and VCO in the top. The blondo was further separated and dried in an oven at 100˚C with different time treatments, namely H1: 3 hours, H2: 6 hours and H3: 12 hours. The dried blondo were then blended for 3 minutes and sieved using a 40 mesh sieve for further analysis.

2.3. Protein, Fat, and Free Fatty Acid Content

Protein and fat content were analysed using the AOAC method [7]. Protein content was analysed with a Kjeldahl method. Free Fatty Acids were measured by adding 10 g of sample to 25 mL of 95% alcohol. The mixture was then heated on a water bath for 10 minutes, shaken, added phenophthalein indicator, and titrated with 0.1 N NaOH [8]. The free fatty acid (FFA) content was calculated by Equation (1).

$$\text{FFA } (\%) = \frac{V\,x\,N\,x\,MW\,FA}{Sample\ Weight\ (g)} x\ 100 \qquad (1)$$

Where:
V = Volume of NaOH titrated in mL
N = Normality of NaOH
MW FA = Molecular Weight of Fatty Acid

2.4. Emulsifying Capacity

The emulsion capacity was measured by mixing 0.2 g of sample in 25 mL of distilled water. The pH of the sample was adjusted to 8 while stirring with a magnetic stirrer for 5 minutes. Furthermore, into 25 ml of the solution added 25 ml of corn oil. The mixture was dispersed in a blender for 1 minute, then centrifuged at 2000 rpm for 10 minutes [9]. Emulsion capacity (EC) was measured by Equation (2).

$$\text{EC } (\%) = \frac{EV}{TV} x\ 100 \qquad (2)$$

Where:
EV = Volume of emulsified mixture
TV = Total Volume of mixture

2.5. Fatty Acid Characteristics

Fatty acid composition of the samples was measured by gas chromatography-mass spectrometry (GC-MS) method [10]. The GC analysis of the fatty acid methyl esters (FAME) was performed using a GC-MS QP 2010 by Shimadzu.

2.6. Statistical Analysis

The experiment was designed in a completely randomized design with various heating treatment and three times replication. The data obtained were then analysed by analysis of variance at 5% significance level.

3. RESULTS AND DISCUSSION

3.1. Protein, Fat, and Free Fatty Acid Content

Protein content of blondo powder treated with different heating times might be seen in Table 1, which showed that the longer the heating time, the lower the protein content. This was probably due to denaturation effect, which resulted in changes in the chemical structure of the protein. In a state where the protein was fully denatured, the protein would only have a primary structure, and no longer have a secondary, tertiary or quaternary structure [11]. In addition, heating would have the potential to cause changes in solubility and hydration, derivatization of amino acid residues, cross-linking, and breaking of peptide bonds, which in turn would lower the protein content.

This study showed that the fat content in blondo decreased with increasing heating time, as shown in Table 1. In general, food processing that use heat would cause fat damage, where the level of damage was highly dependent upon temperature and duration of heating [12]. The heating process on blondo induced the fat to melt, turn into free fatty acids, or even evaporate into other components such as flavour [13].

Table 1. Composition of protein, fat, free fatty acid, and emulsification capacity of blondo prepared with different heating time.

Component of	Heating Time		
	H1 (3 hours)	**H2 (6 hours)**	**H3 (12 hours)**
Protein (%)	31.30 ± 1.13	26.78 ± 0.92	19.89 ± 0.81
Fat (%)	17.94 ± 0.65	14.71 ± 0.82	7.79 ± 1.02
Free Fatty Acid (%)	0.52 ± 0.08	0.60 ± 0.06	0.68 ± 0.05
Emulsification Capacity (%)	77.81 ± 1.78	66.72 ± 1.12	60.03 ± 1.21

Table 2. Fatty acid characteristics of blondo prepared with different heating time.

Fatty Acid of	Heating Time		
	H1 (3 hours)	**H2 (6 hours)**	**H3 (12 hours)**
Lauric (%)	50.45	49.54	48.60
Myristic (%)	18.28	17.42	16,63
Heptadecanoic (%)	8.57	7.82	7.47
Linoleic (%)	8.28	7.05	6.87
Octanoic (%)	8.82	8.82	7.81
Decanoic (%)	7.11	7.04	6.42
Cis-11-eicosenoic (%)	1.07	0.83	0.83
Hexanoic (%)	0.56	0.55	0,50
Butyric (%)	1.06	0.47	0.46

Table 1 also indicated that free fatty acid (FFA) of the products was around 0.52 – 0.68%; and the heating time was not affecting significantly the FFA content. Considering the Indonesian Standard of SNI (01-3741-2002), which stating that the maximum content of FFA was not more than 3%, the results of this study showed that the blondo products were in the acceptable FFA content. The results of this study also showed that increasing heating time would increase slightly the FFA. This might be due to hydrolysis action of the lipase which would be increase with a longer heating time. The hydrolysis would be easier to take place in the fat of short and medium chain, as in this blondo products [8].

3.2. Emulsifying Capacity

Table 1 showed that the emulsion capacity of blondo prepared with different heating time produced various results, with the higher emulsion capacity tended to be higher at a shorter heating time. Emulsion capacity was the ability of a protein solution or suspension to emulsify available fat [9]. Emulsion capacity was due to the ability of the material to absorb water and oil, related to the balance of hydrophobic amino acids and hydrophilic amino acids [14]. Amino acids have polar (hydrophilic) and non-polar (hydrophilic) groups. Therefore, the polar groups in the amino acids would bind to the polar groups in the water and vice versa to form an emulsion. Amino acids which were resulted from protein hydrolysis would be partially absorbed by the oil, and would form emulsion of blondo powder [15].

A higher emulsion capacity at shorter heating time was thought to be due to a higher balance of the relationship between polar and non-polar amino acids, which may have reduced the surface tension. This state would result in a higher emulsion capacity. According to Lam et al. [14], the emulsion formed would be high if the balance of the relationship between hydrophilic and hydrophobic amino acid fractions could reduce the interfacial tension. Meanwhile, the lowest emulsion capacity was at the heating time of 12 hours, which was 60%. The blondo emulsion capacity would decrease with the increasing amount of insoluble protein and increasing disulfide bonds. The treatment with longer heating time could induce protein denaturation which caused an unbalanced relationship between hydrophilic and hydrophobic amino acid fractions. Emulsion capacity was also influenced by solubility, poorly soluble proteins would form deposits on the oil-water surface causing damage to the protein layer.

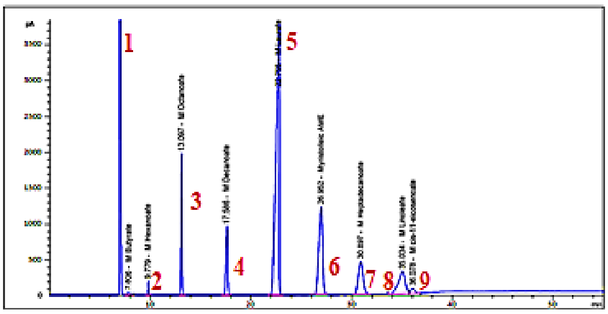
3.3. Fatty Acid Characteristics

As shown in Figure 1, chromatogram of products prepared with variations in heating time of three, six, and 12 hours showed a similar result. It was shown that there were nine peaks of fatty acid components contained in blondo powder prepared at different heating time. They were lauric ($C_{12}H_{24}O_2$), myristic ($C_{14}H_{28}O_2$), heptadecanoic acid ($C_{18}H_{36}O_2$), linoleic ($C_{18}H_{32}O_2$), octanoic ($C_8H_{16}O_2$), decanoic ($C_{10}H_{20}O_2$), cis-11-eicosenoic ($C_{20}H_{38}O_2$), hexanoic ($C_6H_{12}O_2$) and butyric ($C_4H_8O_2$). The composition of their fatty acid produced in heating time of three, six, and 12 hours showed a lightly different results, as indicated in Table 2. Compared with the fatty acid composition in the coconut oil, Odubanjo et al.[10] found that myristic acid was dominant, instead of lauric acid.

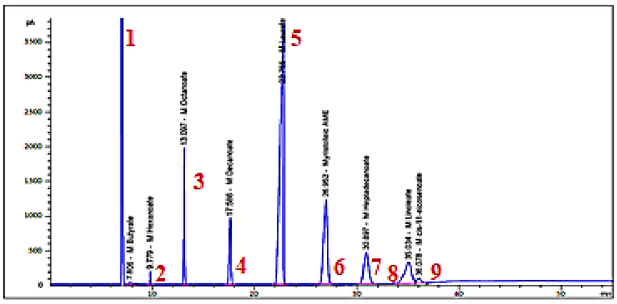
4. CONCLUSION

Blondo as a waste of VCO manufacturing still contained many valuable nutrients, which may be utilized to increase the nutritional value of various processed food products. By changing the blondo into powder form, its application in various food formulations would be increased. The results of this study indicated that varying heating time in the production process would produce various nutritional and physicochemical characteristics. Shorter heating time would result in a product with a higher content of protein, fat, free fatty acids and emulsion capacity.

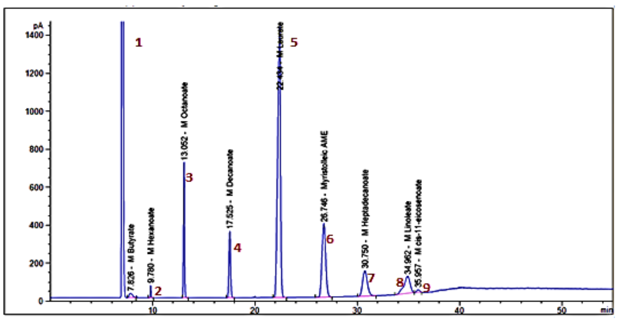
The results of this study in identifying the types of fatty acids contained in this blondo powder indicated that there were nine fatty acid components; namely lauric, myristic, heptadecanoic, linoleic, octanoic, decanoic, cis-11-eicosenoic, hexanoic and butyric. Lauric acid was dominant, and the composition of the fatty acids from each heating treatment was similar and did not show a significant difference.



(a)



(b)



(c)

Figure 1. Fatty acid characteristics of blondo prepared at heating time of (a) 3 hours; (b) 6 hours; (c) 12 hours; legend 1 up to 9 were butyric, hexanoic, octanoic, decanoic, lauric, myristic, heptadecanoic, linoleic, and cis-11-eicosenoic respectively.

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