Chemical changes coconut oil in different fermentation during long time storage

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Abstract. This research aims to know the old fermentation influence the chemical changes during storage on coconut oil. Coconut oil is made with fermented pineapple extract as much as 15%. Coconut oil characteristics compared with the standard National Indonesia No. 01-2902-1999. This research was carried out in the laboratory of Food Analysis Faculty of Agriculture Warmadewa University. This study is a Randomized Design with factorial experiment complete consisting of two factors, namely: factor I, long fermentation that consists of 3 levels, namely 12 hours, 24 hours and 36 hours. Factor II, long storage that consists of 4 levels, namely 0, 1, 2 and 3 weeks. The observed variables against the coconut oil include objective observations i.e. water content, free fatty acids, peroxide, levels of dirt and acid number. The characteristics of coconut oil during storage tend to change but still meet Indonesian National Standard No. 01-2902-1999. From the results of research on the long fermentation 12% with prolonged storage 0 weeks gives the characteristics of the best oils than any other treatment that is the average value of water content, the level of dirt, free fatty acids, peroxide and acid number respectively i.e. 0.17%, 1.56%, 0.53%, 1.45% and 2.20%. During the storage of chemical changes tend to occur on coconut oil. The value of the average water content, the level of dirt, free fatty acids, peroxide and acid number highest obtained on treatment of fermentation 36 hours and long storage of 3 weeks in a row with a value of 0.50%, 3.70%, 0.85%, 2.51% and 4.38%.

1. Introduction

Traditional Coconut Oil which has a disadvantage of high peroxide numbers which will trigger the rancidity process. This is because the heating process is quite long [1]. The process of making coconut oil also requires a fairly long process and consumes a lot of fuel. This is what causes the price of coconut oil to tend to be expensive. In addition to affecting the cost of production, heating long enough can affect the quality of the oil, especially the reduction in the nutritional value of oil. Long heating in the manufacture of oil by traditional means also triggers the presence of acrolein compounds in oils that are harmful to the body.

Therefore, it is necessary to improve the processing of coconut oil which does not use sedimentary and bleaching chemicals, namely by fermentation. Treatment with fermentation is expected not to require chemicals and avoid the formation of trans fatty acids. The advantages of extracting coconut oil by fermentation are practical, energy efficient, low residue, low acid numbers and free of cholesterol-inducing compounds [2]. One of the fermentation treatments that can be done in making coconut oil is
the addition of enzymes. The enzyme that can be utilized in the process of making coconut oil is the enzyme Bromelain which is sourced from pineapple fruit.

The Bromelain enzyme is a type of protease enzyme that is able to hydrolyze peptide bonds in proteins into smaller molecules, namely amino acids so that the body is easily digested. Bromelain enzymes are found in all pineapple plant [3]. The use of Bromelain enzymes in the manufacture of coconut oil is basically still in the form of liquid (fruit juice). The making of coconut oil enzymatically in this case using the Bromelain enzyme is done by the wet method (wet rendering) where the enzyme will degrade the protein component and break down the coconut cell wall so that the oil is easier to separate from the water.

In the research Widnyana, pineapple extract produced the highest quality oil [4]. Based on the research of Janurianti, the concentration of Bromelain enzyme used in the fermentation of coconut oil is 15% with a duration of 12 hours’ fermentation [5]. The quality of coconut oil produced meets Indonesian national standards. Different fermentation times are thought to tend to affect the quality of coconut oil because fermentation also increases peroxide numbers. According to Winarno, the oil oxidation reaction begins with the formation of free radicals caused by factors that can accelerate reactions such as light, heat energy, metal catalysts and enzymes so that long fermentation processes can increase peroxide numbers [6].

Based on the background above, the researchers wanted to conduct a study on the effect of fermentation time on coconut oil storage on the chemical quality of coconut oil. This study aims to determine whether there is an effect of fermentation time on coconut oil storage. This research is to learn more about the effects of fermentation on the quality of coconut oil during storage.

2. Materials and methods
The research was conducted at the Food Analysis Laboratory of the Faculty of Agriculture, Warmadewa University. The study was conducted for 5 months, from November 2018 to March 2019. The raw material used in this study is medium-sized coconut fruit. The pineapple used in this research is Pineapple Honey. Other materials used are water. The chemicals used in the analysis are aquades, 0.1 N KOH solution, pineapple, 0.1 N NaOH solution, 0.1 N sodium thiosulfate, 96% alcohol, phenolphthalein indicator, chloroform, glacial acetic acid, petroleum ether, saturated KI solution, 0.5 N HCL solution, 1% starch indicator, filter paper and whatman paper No.42. The tools used in the process of making coconut oil in this study are pans, plastic washbasins, pans, spoons, stoves, stirrers, plastic hoses, plastic funnels, filter cloth and thermometers. While the tools used for analysis are beaker, funnel, stirring rod, measuring cup, blender, erlenmeyer, statif, clamp, oven, porcelain cup and drop pipette.

This research is a factorial experiment with Randomized Block Design (RBD). Grouping is based on different sample processing times. This study consisted of 2 (two) factors, namely: factor I, duration of fermentation consisting of 3 levels, namely 12 hours fermentation time (L1), 24 hours fermentation time (L2) and 36 hours fermentation time (L3). Factor II, storage time which consists of 4 levels, namely storage of days 0, 1, 2 and 3 weeks. Each treatment was repeated 2 times to obtain 24 experimental units. Data obtained from objective tests were processed by analysis of variance (F test), if the results showed a significant / very real effect on the characteristics of the oil then proceed with the 5% and 1% LSD test.

3. Results and discussion
Traditional coconut oil which is modified by the fermentation process and given treatment according to the design of the study is objectively analyzed with parameters of water content, impurities, free fatty acid levels, peroxide numbers and acid numbers still in accordance with the Indonesian National Standard (SNI). Interactions between fermentation time and storage time showed a significant effect (P <0.05) on impurities and acid numbers, and did not significantly (P>0.05) on the variable moisture content, free fatty acid levels and peroxide levels.
3.1. Water content
The highest moisture content is at 36 hours fermentation treatment with 3 weeks storage which is 0.50%. The average moisture content of coconut oil can be seen in Table 1. Coconut oil moisture content still meets the Indonesian National Standard (SNI) where the coconut water content is max. 0.5%. In the treatment of fermentation, the highest water content was obtained at 36 hours fermentation treatment which was 0.49% which was significantly different from the other treatments.

The fermentation time is seen to be able to increase the water content of coconut oil, this is due to the content of ingredients such as more protein and enzymes. This is evidenced by the color of the yellow fermented oil. The presence of proteins and enzymes is likely to bind water from the environment [7]. Water content is related to the hydrolysis reaction of fat. If there is water in the fat or oil, the oil will be hydrolyzed to produce free fatty acids and glycerol. While for the long storage time, the highest coconut oil moisture content was obtained at the 3rd week storage, which was 0.38% which was not significantly different from the water content at the 1st and 2nd high storage but not significantly different from the 0th week storage. Storage does not have a significant effect on the moisture content of coconut oil because coconut oil is placed on a closed bottle.

Table 1. Average water content (%) of coconut oil in the treatment of old fermentation and storage length.

| Treatment        | Week 0 | Week 1 | Week 2 | Week 3 | Average |
|------------------|--------|--------|--------|--------|---------|
| Fermentation 12 hour | 0.17   | 0.22   | 0.23   | 0.24   | 0.22 c  |
| Fermentation 24 hour | 0.37   | 0.39   | 0.39   | 0.41   | 0.39 b  |
| Fermentation 36 hour | 0.48   | 0.49   | 0.49   | 0.49   | 0.49 a  |
| Average          | 0.34 b | 0.37 ab| 0.37 ab| 0.38 a |         |

Information:
- The average value followed by the same letter in the same row and column shows a non-significant difference (P>0.05).
- The mean values followed by different letters on the same row and column show significant differences (P <0.05) to very significant (P <0.01).

3.2. Impurities
The highest levels of impurities in coconut oil were obtained at 36 hours fermentation treatment with a storage period of 2 weeks. The average level of impurities of coconut oil can be seen in Table 2. Coconut oil manure did not meet the requirements of the Indonesian National Standard (SNI) in 1999.

Table 2. Average levels of impurities (%) of coconut oil in the treatment of long fermentation and duration of storage.

| Treatment        | Weeks |
|------------------|-------|
|                  | 0     | 1     | 2     | 3     |
| Fermentation 12 hour | 1.56  | 2.52  | 2.92  | 3.41 a |
|                   | C     | B     | AB    | A     |
| 24 hour           | 2.62  | 2.71  | 3.23  | 3.33 a |
|                   | B     | B     | AB    | A     |
| 36 hour           | 3.36 a| 3.61 a| 3.63 a| 2.47 b |
|                   | B     | B     | A     | B     |
| LSD 0.05          | 0.58  |

Information:
- The average value followed by the same letter in the same row and column shows a non-significant difference (P>0.05).
- The mean values followed by different letters on the same row and column show significant differences (P <0.05) to very significant (P <0.01).
The level of impurities based on SNI was a maximum of 0.05%, while the content of impurities of coconut oil made ranged from 1.56% - 3.63%. The high levels of impurities in 36-hour fermentation are due to the longer fermentation, the more protein, carbohydrates and polypeptides that are degraded to produce a lot of material dissolved in oil. The high level of impurities in coconut oil is due to the process of making traditional coconut oil using ordinary filter cloth so that there is still a lot of fine dirt that escapes.

3.3. Free fatty acid
The free fatty acid content of coconut oil obtained from the old fermentation treatment and storage time fulfills the requirements of the Indonesian National Standard (SNI) Number 01-2902-1999, where the free fatty acid content in SNI requirements is a maximum of 5%.

The highest free fatty acid content was obtained at 36 hours fermentation treatment and 3 weeks storage which was 0.85%. The average level of free fatty acids in oil can be seen in Table 3. The longer fermentation tends to increase the levels of free fatty acids due to the hydrolysis by enzymes. Storage is also able to increase the levels of free fatty acids because the longer it is stored, the longer the contact with oil and water can break down fatty acids in oil. Free fatty acids are formed because of the hydrolysis process. Hydrolysis of oil can be caused by the amount of water contained in oil [8].

Table 3. Average levels of free fatty acids (%) coconut oil in the treatment of long fermentation and storage length.

| Treatment          | Week 0 | Week 1 | Week 2 | Week 3 | Average |
|--------------------|--------|--------|--------|--------|---------|
| Fermentation 12 hour | 0.53   | 0.70   | 0.71   | 0.80   | 0.68 b  |
| Fermentation 24 hour | 0.54   | 0.70   | 0.65   | 0.69   | 0.64 b  |
| Fermentation 36 hour | 0.80   | 0.78   | 0.80   | 0.85   | 0.81 a  |
| Average            | 0.62 b | 0.73 a | 0.72 a | 0.78 a |         |

Information:

- The average value followed by the same letter in the same row and column shows a non-significant difference (P> 0.05).
- The mean values followed by different letters on the same row and column show significant differences (P <0.05) to very significant (P <0.01).

3.4. Peroxide numbers
The peroxide value of coconut oil obtained from the treatment of long fermentation and storage time meets the requirements of the Indonesian National Standard (SNI) in 1999, which is a maximum of 5%. Peroxide is the most important value for determining the degree of damage to oil or fat. Unsaturated fatty acids can bind oxygen to their double bonds to form peroxide [9]. The average oil peroxide number can be seen in Table 4. The highest peroxide number was obtained in 24-hour fermentation with a storage period of 3 weeks. Peroxide numbers formed from oil oxidation reactions begin with the formation of free radicals caused by factors that can accelerate reactions such as light, heat energy, metal catalysts and enzymes. Free radicals with oxygen will form active peroxides which can form hydroperoxide which is very unstable [5].

Table 4. Average peroxide level (%) of coconut oil in the treatment of old fermentation and storage time.

| Treatment          | Week 0 | Week 1 | Week 2 | Week 3 | Average |
|--------------------|--------|--------|--------|--------|---------|
| Fermentation 12 hour | 1.45   | 1.82   | 2.17   | 2.16   | 1.90 c  |
| Fermentation 24 hour | 2.39   | 2.49   | 2.50   | 2.63   | 2.50 a  |
| Fermentation 36 hour | 1.98   | 2.38   | 2.46   | 2.51   | 2.33 b  |
| Average            | 1.94 c | 2.23 b | 2.37 a | 2.43 a |         |
3.5. Acid numbers

Acid numbers are important, which is to measure the amount of free fatty acids found in oil or fat [9]. The average value of acid oil can be seen in Table 5. The oil acid number is quite high because in the process of making oil it uses pineapple extract containing organic acids. The acids contained in pineapple are citric acid, malic acid, and oxalic acid. The most dominant type of acid is 78% citric acid from total acid [10]. In SNI there are no requirements for the level of acid number of coconut oil. The acid number of coconut oil ranges from 1.0-10.0% [4,11]. Based on observations of oil acid numbers ranged from 2.20 to 4.42%. The duration of fermentation and the length of storage of oil tends to increase the acid number because pineapple extract has a longer direct contact with oil so that the organic acids in pineapple extract are more soluble in coconut oil.

Table 5. Average (%) acid numbers of coconut oil in the treatment of old fermentation and storage length.

| Treatment | Weeks | 0 | 1 | 2 | 3 |
|-----------|-------|---|---|---|---|
| Fermentation 12 hour | | 2.20 | c | 2.29 | C | 2.33 | C | 2.33 | a |
| | | B | AB | A | A | | | | |
| Fermentation 24 hour | | 3.42 | b | 3.28 | B | 3.50 | B | 3.62 | a |
| | | B | C | AB | A | | | | |
| Fermentation 36 hour | | 4.28 | a | 4.36 | A | 4.42 | A | 2.92 | b |
| | | B | AB | A | C | | | | |
| BNT 0.05 | | 0.12 |

4. Conclusions

Based on the results of the study it can be concluded that the fermentation time and storage time showed a significant effect on the amount of impurities and acid numbers, and did not significantly influence on variable moisture content, free fatty acid content and peroxide levels. In general, coconut oil made with pineapple extract fermentation for 12, 24 and 36 hours with a storage period of 3 weeks still meets the 1999 Indonesian national standard (SNI) [12] except for impurities. The high level of impurities is caused because the filter cloth used is ordinary filter cloth so that a lot of dirt escapes into the oil. During storage, chemical changes tend to occur in coconut oil. The average value of water content, impurities, levels of free fatty acids, peroxide levels and highest acid numbers were obtained at 36 hours fermentation treatment and 3 weeks storage time with values of 0.50%, 3.70%, 0.85 %, 2.51% and 4.38%.

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