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The Effect of Fermentation Time and Mass Variation of Saccharomyces Cerevisiae on the Characteristics of Virgin Coconut Oil from the Fermentation Process of Coconut Milk

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ABSTRACT

The many advantages of processed coconut products are shown by virgin coconut oil. Extraction, centrifugation, and fermentation are the three main methods for producing virgin coconut oil. In this experiment, baker's yeast (Saccharomyces cerevisiae) was used to ferment virgin coconut oil. The purpose of this research is to identify the properties of virgin coconut oil as a function of fermentation duration and mass variation of Saccharomyces cerevisiae. According to this research, separating the water from the milk of coconuts is the first step in producing virgin coconut oil. Fermentation lengths of 12, 18, and 24 hours are used to combine skimmed cream with Saccharomyces cerevisiae, with amounts ranging from 0 to 2 grammes. Iodine, peroxide, and acid quantities are then determined by analysing the virgin coconut oil that is produced. Research shows that while making virgin coconut oil from coconut milk, the amount of time yeast is left to ferment and the mass of Saccharomyces cerevisiae do not correlate with the iodine number. The peroxide number for all variables of fermentation duration and yeast mass is two meq/kg. The acid number tends to increase with the length of fermentation, with the highest value being 0.6% at 24 hours of fermentation time and the yeast mass of Saccharomyces cerevisiae 1.5 grams.

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1. INTRODUCTION

Coconut (*Cocos nucifera L.*) is a plant that can be used by humans, from the tops of the leaves to the roots. Coconut belongs to the palm tribe (aracaceae) and is a plant that has many benefits and thrives in tropical regions such as Indonesia. Coconut palms grow up to 30 meters, producing 75 fruits per year with favourable environmental conditions[1]. In the city of Sorong, Southwest Papua Province, coconuts thrive along coastlines as well as plantations. Coconut production in West Papua Province in 2019 was 16,169 yon, an increase of 2.62% compared to 2018. So, the diversification of coconut products is needed to increase the usefulness of coconuts in the community.

At the beginning of 2022, Indonesia experienced a scarcity of palm oil products, which are the people's main choice in processing fried foods. This causes people to start looking for other alternatives to the use of goring oil, one of which is coconut oil. Coconut oil can be made on a household scale by cooking coconut milk into coconut oil and blondo. However, this process requires a long time and a lot of fuel[2]. Besides being able to be obtained into cooking oil, old coconut milk can be processed into *Virgin Coconut Oil* (VCO), which does not require high temperatures in the manufacturing process, otherwise known as *the wet coconut process*.

Unlike in the manufacture of coconut oil, VCO maintains the distinctive aroma of coconut so as to produce more fragrant dishes. VCO contains higher saturated fatty acids than palm oil, so it tends to be more stable in structure [3]. VCO is also considered healthier, with antioxidant content, acts as a probiotic, and can play a role in weight loss. In addition, VCO can also be used in skin and hair care.

The process of making VCO can be done in several ways, namely enzymatic, microbial fermentation, acidification, centrifugation, and fishing [3]. Virgin coconut oil is extracted from fresh coconut flesh or dried coconut meat. The oil content in copra is generally 60-65%, while the oil content in fresh coconut flesh is around 43% [4]. Mechanically, by spinning at high speeds, the centrifugation process separates the fat-protein linkages in coconut milk. Water and oil will naturally separate after centrifugation since their specific gravities are different [5].

In this study, a fermentation process was used to make VCO by using baker's yeast (*Saccharomyces cerevisiae*) as a starter in the fermentation process of old coconut milk to become VCO. Baker's yeast is used because, in the form of emulsions, it can produce enzymes that play a role in converting glucose into alcohol. The resulting alcohol plays a role in breaking down the coconut milk emulsion to produce oil [6]. The advantages of fermentation demtode are energy saving, ease of taking the oil produced, and the quality of the oil produced can be maintained by adjusting the ratio of raw materials with *Saccharomyces cerevisiae* used [7].

2. METHOD

The necessary ingredients are aged coconut meat, water, baker's yeast (*Saccharomyces cerevisiae*), chloroform, Wijs solution, Potassium Iodide, NaOH, Sodium Thiosulfate, amylum indicator, phenolphthalein indicator, 95% ethanol. Old coconut meat obtained at the market in Sorong City is shredded and squeezed by adding enough water to produce coconut milk. The next step in making coconut milk cream is to let the milk sit for two hours. Next, the water is drained from the coconut milk and its cream is collected. 100 grammes of coconut milk cream with baking yeast starting at concentrations of 1, 1.5, and 2 grammes, respectively. After that, the fermentation process is carried out anaerobically for 12 hours, 18 hours and 24 hours.

Test Number of Iods

The iodine number was measured by weighing 5 grams of coconut oil sample and then put into a lidded Erlenmeyer. Then, for 30 minutes while shaking every so often, 10 millilitres of chloroform and 25 millilitres of Wijs reagent were kept in a dark room. Next, 50 ml of equates, 2 ml of amylum, and 10 ml of 15% KI solution were used. Next, titrate with 0.05 N sodium thiosulfate until the blue tint goes away [8]. The formula for the iodine number, which is grammes of absorbed iodine per 100 g, is:

$$Iodine\ number = \frac{12{,}69\ \times T\ (V_3 - V_4)}{m}$$

Information:
T = Normality of standard solution of sodium thiosulfate
V3 = Sodium thiosulfate solution volume needed for blank titration in millilitres
V4 = Sample titration volume for ntarium thiosulfate solution
m = sample weight (grams)

Test Number of Peroxide

The peroxide number was measured by weighing 0.3 - 5.0 grams of oil samples into a 300 ml. Mix 20 ml of glacial acetic acid, 25 ml of 95% ethanol, and 55 ml of chloroform in 30 ml of Erlenmeyer's solution. Before adding aquadest titrate, which is a standard solution of 0.02 N sodium thiosulfate with starch solution as an indicator, add I gr KI and let it sit in a dark spot for 50 ml. Determine the sample's peroxide number and set up blanks [9].

The number of peroxides is expressed in milligrams of the equivalent of active oxygen per kg, using the formula:

$$Peroxide\ number\ \left(\frac{mgrek}{kg}\right) = \frac{(V_0\ -\ V_1\ ) \times T}{m} \times 1000$$

Information:
V0 = Volume of sodium thiosulfate solution for blanks in ml
V1 = Volume (ml) of sodium thiosulfate solution for sample
T = Normality of standard solution of sodium thiosulfate used

M = Example weight in grams

Test the Number of Acids

Weigh 2-5 grams of oil sample into Erlenmeyer 250 ml, then add 50 ml of 95% neutral ethanol. Add 3 – 5 indicators of Phenolpthalein and titrate with a standard solution of 0.1 N NaOH until the pink colour remains (unchanged for 15 seconds) [9]. The acid number is expressed as mg KOH/gram of fat using the formula:

$$Free\ fatty\ acid\ levels = \frac{M\ \times V \times T}{10\ m}$$

Information:
V = Volume of NaOH required in titration (ml)
T = normality NaOH
m = sample weight in grams
M = Molecular weight of fatty acids

In coconut oil testing, the acid number is calculated as lauric acid, which has a molecular weight of 200.

3. RESULTS AND DISCUSSION

The outward manifestations of virgin coconut oil include a clear aroma of coconut, the absence of rancidity, a flavour profile typical to coconut oil, and an obvious hue. Virgin coconut oil made to Indonesian National Standard (SNI) standards looks, smells, tastes, and looks the same as other brands.

Effect of Fermentation Duration and Yeast Mass of Saccharomyces cerevisiae on Iodine Number

The iodine number may be used to determine how unsaturated the fatty acid components of the fat are. Adding yeast and adjusting fermentation time had no discernible impact on the outcome of the whole treatment, according to linear regression analysis ($p > 0.05$). Based on Table 1, the average iodine number resulting from the influence of fermentation duration and yeast addition is 4.16 – 4.57, where this value is in accordance with the standard set by SNI for iodine number in virgin coconut oil, which is 4.10 – 11.0 mgrek / kg.

Table 1. Effect of Fermentation Duration and Yeast Mass of *Saccharomyces cerevisiae* on Iodine Number

Fermantation Time	**Massa Ragi *Saccharomyces cerevisiae***			
	0 gr	**1 gr**	**1,5 gr**	**2 gr**
12 hours	4,31	4,48	4,40	4,19
18 hours	4,42	4,37	4,57	4,26
24 hours	4,16	4,29	4,29	4,25

Oils with low iodine numbers tend to have low levels of unsaturated fatty acids. Oil contains saturated molecules and unsaturated linkages formed by iodine and unsaturated fatty acids. A measure of the amount of double bonds is the quantity of iodine bound [10].

The Old Influence of Fermentation and Mass Yeast Saccharomyces cerevisiae on the Number of Peroxide

Peroxide number is the amount of peroxide present in the sample, expressed in terms of milliequivalent active oxygen per kg, which oxidizes potassium iodide under treatment conditions such as in peroxide number testing (SNI). It is associated with rancidity in the oil and a decrease in the quality and shelf life of fat [11].

Table 2. Effect of Fermentation Time and Yeast Mass of *Saccharomyces cerevisiae* on Peroxide Number

Fermantation Time	**Massa Ragi *Saccharomyces cerevisiae***			
	0 gr	**1 gr**	**1,5 gr**	**2 gr**
12 hours	2	2	2	2
18 hours	2	2	2	2
24 hours	2	2	2	2

In this study, it was found that in all variables, both the length of fermentation time and the mass of yeast Saccharomyces cerevisiae used produced the same peroxide number, which is 2 meq/kg. The value of this peroxide number is still in accordance with the SNI standard for virgin coconut oil, which is 2 meq/kg. Traditional coconut oil products had a higher peroxide level when fermented for longer, according to Patty's study. This is due to the fact that prolonged fermentation exposes the coconut oil to oxygen, which increases the concentration of unsaturated fatty acids. Consequently, there will be an upsurge in the production of free radicals, which are subsequently transformed into hydroperoxide [12]. Free radicals are produced in the first step of oil oxidation reactions by means of reactants that may accelerate the process, including heat, light, enzymes, and metal catalysts. In the presence of oxygen, free radicals may generate active peroxides, which in turn can produce unstable hydroperoxides.

Effect of Fermentation Time and Yeast Mass of Saccharomyces cerevisiae on Acid Number

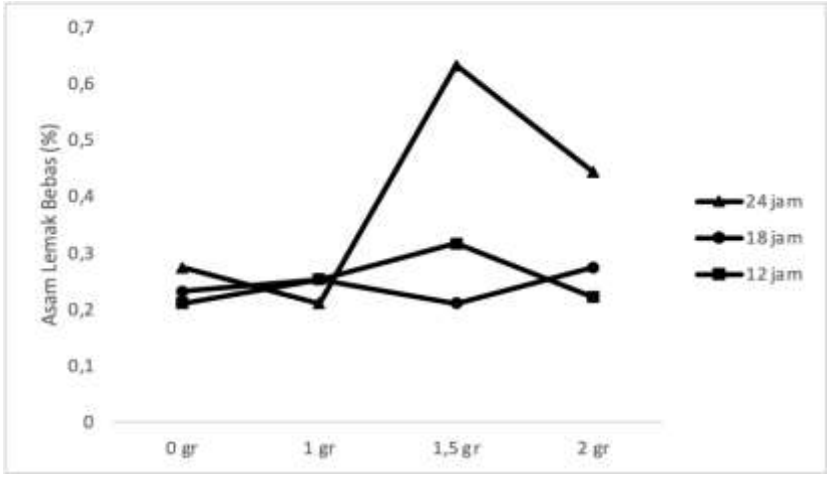

Figure 1. The acid number of virgin coconut oil

Findings for the amount of free fatty acids varied between 0.2% and 0.6%; adding Saccharomyces cerevisiae yeast to the mixture during the first 24 hours of fermentation increased the amount of free fatty acids by up to 1.5 grammes compared to other yeasts and the same amount of time.

Free fatty acids can be benthic since the oil is still in plant tissues due to the presence of lipase enzymes that can hydrolyze neutral fats (triglycerides). But in living organisms, enzymes are generally in an inactive state or state because there are still interactions between cells. In organisms that have died, the mechanism of the cells gets damaged so that the lipase enzyme starts working and damages the fat molecules. The speed of hydrolysis of lipase enzymes present in tissues is relatively slower at low temperatures and will be more intensive under suitable conditions. Their cell structure has been damaged in coconuts that have been shredded so that lipase enzymes begin to work, damaging fat molecules [13]. According to research, the concentration of free fatty acids in oil increases as fermentation duration increases [5]. As a measure of oil degradation caused by hydrolysis in the presence of water and lipase enzyme activity, free fatty acids are an important consideration; hence, a lower free fatty acid content indicates higher oil quality. There should be no more than 0.2% free fatty acids in virgin coconut oil, according SNI.

4. CONCLUSION

Preparing virgin coconut oil from coconut milk to iodine number does not depend on the quantity of the yeast Saccharomyces cerevisiae or the length of fermentation. For all values of yeast mass and fermentation time, the peroxide number is 2 meq/kg. At 24 hours of fermentation and 1.5 grammes of Saccharomyces cerevisiae yeast mass, the acid number reaches 0.6 percent, and it tends to rise with fermentation duration.

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