

Manufacture of solid soap based on crude papain enzyme and antioxidant from papaya

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Abstract. The uncontrolled formation of free radical formation released to the air especially in the high urban area lead to resulting negative effect on the air quality. This condition is consequently made the human skin continuously contacts to those radicals and may cause a skin diseases. Therefore, one way to overcome this problem is to manufacture a soap formulation which provide a new function of its ingredient as radical scavenger or to keep the skin in health. Papain is a plant proteolytic enzyme for the cysteine protease enzyme which found naturally in papaya (*Carica papaya*) manufactured from the latex of raw papaya fruits. Proteolytic enzyme has a function which is able to break down organic molecules made of amino acids, known as polypeptides. In this research crude papain enzyme is developed into active ingredient of solid soap formulation with the antioxidant originated from fresh papaya to enhance the useful soap for human skin health. This study aims to produce a solid soap formula that is safe for the skin, meets the standard of SNI 1996 and tested the benefits in the addition of crude papain enzymes. The result showed that Formula IV is a formula soap that meets standard SNI 1996 and have highest antioxidant activity among other formulas indicated by the value of IC₅₀ is 13,657 ppm. Compared soap with positive control (without enzymes) than negative control (with enzyme) has value of percentage dirt removal higher than positive control, it is 19% with measurement absorbance of wash water substrate and 32% measured by mass substrate after washed.

1. Introduction

As protection of the body from the outside, the skin has various problems such as dry skin, premature aging, chronic diseases like skin cancer. This can be caused by air pollution resulting in many free radicals, exposure ultraviolet, and also less care for cleanliness. The main treatment for maintaining skin health is to use soap regularly. Soaps are sodium or potassium salts of fatty acids derived from vegetable oils or animal fats [1]. The use of chemicals will provide side effects on sensitive skin, while soaps with natural ingredients will provide the maximum nutrition on the skin.

Papain enzymes found in papaya's soap have high activity in the breakdown of proteins into peptides and amino acids. This makes papain can be used as an active ingredient in the manufacture of skin cleanser because it can dissolve dead cells that are attached to the skin and difficult to get rid of in a physical way. Antioxidants are substances that the body needs including the skin to neutralize free radicals and prevent damage caused by free radicals. In this study we make solid soap containing antioxidant as crude from papaya fruit and crude enzyme papain from latex of papaya.



2. Material and Methods

2.1. Manufacture Solid Soap

The raw material for solid soap were coconut oil, NaOH, water, citric acid, glycerin, papaya fruit, crude papain enzyme. The oil mixed gently with lye solution and kept warm at ambient temperature then placed in the stainless steel pot soap, stirred frequently for 5-10 min. while stirring the mixture added the other ingredients and next pour the solution into the moulds in the ambient temperature for 24 hours until solution change into solid. Formulation of soap presented in Table 1 below

Table 1 Solid soap formulation

Ingredients	Formula (%w/v)					
	I	II	III	IV	V	VI
Coconut Oil	55	48	42	42	55	48
NaOH	7	6	6	6	7	6
Water	21	19	17	17	21	19
Stearic Acid	0.8	0.7	0.6	0.6	0.8	0.7
Glycerin	1.5	1.2	1.1	1	1.5	1.2
Papaya	15	25	30	30	15	25
Perfume	0.5	0.5	0.5	0.5	0.5	0.5
Crude enzyme papain	0.5	1	1.5	1.5	1	0.5

2.2. pH Determination

The pH was determined using pH meter and prepare 10% soap solution with distilled water. The electrode of the pH meter was inserted into solution after calibration with buffer solution. The pH reading was recorded.

2.3. Foam Ability test

Around 1 gram of the sample soap was added to 20 mL measuring cylinder containing 10 mL of distilled water. The mixture was shaken using vortex vigorously so as to generate foams. After shaking for about 30 sec, the measuring cylinder was allowed to stand for 5 min (t_5). The height of the foam in the solution was then measured and recorded.

2.4. Fatty Acid Determination

10 gram of sample soap was added into 100 mL distilled water in the 250 mL erlenmeyer flask and heated in water bath. Subsequently the sample soap titrated with H_2SO_4 20% using methyl orange indicator until the solution become red. Stir solution to homogenized and heated until two phased was formed. Next, get 10 gram micro paraffin (beeswax) into that solution and heated for an hour until the solution was formed in one phased and then cooling the erlenmeyer flask in the water bath quickly. Mixture of beeswax (wax cake) and fatty acid was change into solid state in the flask, and measured the weight of sample (1). Fatty acid can be calculated with the formula :

$$Fatty\ acid = \frac{m_w - m_b}{m_s} \times 100\% \quad (1)$$

Where m_w is weight of wax cake, m_b is weight of micro paraffin beginning, m_s is weight of sample soap.

2.5. Alkali Free Determination

First step to determination alkali free was to prepare 100 mL alcohol 96% into flask and then added indicator phenolphthalein and subsequently titrate with KOH 0,1 N until appear pink colour in solution. 5 gram of sample soap was added into alcohol neutral solution and also boiling stone or flakes of porcelain it use to avoid an explosion. Second step boiled until soluble solution dissolves for 30 minutes. If it doesn't contain alkali (colour of solution not pink), cooled it to 70°C and titrates with

0.1N KOH until pink colour stand for 15 seconds. Next step titrated the solution with 0.1N HCL in the alcohol with micro burette until the pink colour disappears. Alkali free can be calculated in formula

$$\text{Alkali free} = \frac{V \times N \times 0.04}{W} \times 100\% \quad (2)$$

Where V is volume of HCL, N is Normalitas of HCL, W is mass of sample soap, 0.04 is mass of relative NaOH

2.6. Specific Density Determination(25°C)

1 mL of the sample soap was measured in a clean micro tube and stand for a while into incubation until get 25°C (room temperature). The weight of both sample soap and the micro tube was also measured then the weight of the sample soap was obtained by subtracting the weight of micro tube from the weight of both the sample soap and measuring micro tube. The specific density of the sample soap is then obtained using: stand for 5 min (t_5) and 30 min (t_{30}). The height of the foam in the solution was then measured and recorded

$$\text{Density (25°C)} = \frac{W_1 - W_0}{V_0} \times 100\% \quad (3)$$

Where W_1 is weight of measuring micro tube and the sample, W_0 is weight of measuring of micro tube, and V_0 is volume of the sample used. stand for 5 min (t_5) and 30 min (t_{30}). The height of the foam in the solution was then measured and recorded.

2.7. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

Various amounts of samples dissolved in methanol and have concentration 500, 1000, 1500, 2000, and 2500 ppm each sample were added to DPPH solution 100 ppm. After 30 min of incubation at room temperature, the absorbance at 516 nm was measured using spectrophotometer UV-VIS. Percent inhibition of free radical DPPH was calculated as follows:

$$\text{Antioxidant activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\% \quad (4)$$

The quality of radical scavenging property of the soap was determined by calculating IC_{50} . The IC_{50} value is the concentration of each sample of soap required to scavenge the DPPH radical to 50% of the control. The concentration of sample soap necessary to decrease the initial DPPH calibration curve. Radical scavenging activity of sample soap was calculated as IC_{50} . The lower IC_{50} value indicates the higher DPPH radical scavenging activity.

2.8. Effectiveness in Cleaning

First step is prepare clean white fabric as a substrate 5cm x 5cm. The dirt is formed by protein from egg and milk mixture. Paste the dirt on the substrate by dipping for a minute and drying it. Wash the substrate with control positive soap (without crude enzyme) and control negative soap (with crude enzyme) for 2,4,6,8,10 minutes using stirrer. Performance test which aims for effectiveness in cleaning consist of 2 methods there are removal dirt test which aims to know the capability of the detergent to remove dirt and by measuring the absorbance of wash water substrate using spectrophotometer UV-VIS at wavelength 270 nm. For removal dirt test

$$\text{removal dirt (\%)} = \frac{m_d - m_c}{m_d} \times 100\% \quad (5)$$

Where m_d is mass of dirt substrate and m_c is mass of clean substrate after washing with soap sample.

3. Result and discussion

3.1. Soap Analysis

Table 2 present the quality criteria of soap based on BSN (*Badan Standarisasi Nasional*) which is safe for human health skin.

Table 2 Soap quality terms

No	Parameters	
1	Fatty acid (%)	>70
2	Alkali free (%)	
	as KOH (liquid soap)	Max 0,22
	as NaOH (solid soap)	Max 0,1
3	Specific density (25°C)	1,01-1,1
4	pH	8-11

Sources (1)

Saponification occurs when, first, three molecule of sodium hydroxide (NaOH) are dissolved in water (H₂O) and are splits apart, which results in three sodium ions (Na) and three hydroxyl groups (OH). Second, a triglyceride (fat) molecule (C₃H₅(COOR)₃) is hydrolyzed reaction, which results three fatty acid tails (COOR). Third, the hydroxyl groups (OH) all bond to the free glycerol (C₃H₅) to form a molecule of glycerin (C₃H₅OH₃). Fourth, the three fatty acids (COOR) each bond with one of the three sodium ions (Na) to form three molecules of soap (3NaCOOR) (2). The result saponification was measured in table 3 all sample (F) and physiochemical properties some parameter.

Table 3 Result of physiochemical properties of solid soap for all formula

Sample	pH	Fatty acid (%)	Alkali free (%)	Specific density (gr/mL)	Foam ability (t ₅) (%)
F I	10.3	87	0.110	1.0873	82
F II	10.4	78	0.219	0.9120	83
F III	10.3	86	0.217	1.0619	86
F IV	10.35	79	0.108	1.0595	78
F V	9.95	78	0.109	1.0278	95
F VI	10.2	82	0.108	1.0271	67

As measured in table 3 the high pH content indicating high percentage amount of unspecified and unsaponifiable matter due to incomplete alkaline hydrolysis. The pH value measurement of the whole formula has an alkaline formed because the amount of alkali present in the soap preparation affects the pH value. The basic ingredients used are NaOH, one of the strongest bases, which play an important role in producing saponification reaction with fatty compound. Based on criteria from (1) all formula enter the threshold.

Fatty acids composition of oil used in soap formation can also determine the nature and stability of leather produced by different soaps, it was reported that, lauric acid, myristic acid, which are all saturated fatty acids produce soap with fatty leather, higher cleansing power but of low stability (3). The principle of measuring the amount of fatty acids containing in the soap is by breaking the bonds between fatty acids with sodium in soap using strong acid, then extract it with micro paraffin wax to form a cake containing mixture micro paraffin wax, the amount of fatty acids, and mineral oil may exist (1). SNI establishes a standard amount of fatty acid of at least 70% which means that the ingredients added as a filler in the soap should be less than 30%. With the aim to be able to accelerated the dirt removal form of oil or fat when used as ingredients soap.

Alkali free is alkali that is not bound as a compound at the time of making soap. Table 3 shown that there are two formula that doesn't meet the range of requirement that determined by SN, it is cause due to the excessive addition of alkali in the preparation and the lack of the amount of fatty acids in the soap. Free alkali that exceeds the standard will cause skin irritation and it will also cause dry skin (4). Oil with low density is an indication that it contains low molecular weight fatty acid, likewise it will have high saponification value which makes it suitable for soap production. The density for formula II doesn't meet range standard BSN, which has a papaya content of 15% and a crude papain enzyme 0.5%. The result can be due to the soap composition of the unbalanced formula so it is not included in the SNI standard range. Most ingredients such as sugars and salts may cause increased density, but density may also decrease if there is fat or ethanol in solution.

Most consumer like cleaning products that have abundant foams, but the amount of foam in the cleaning products due to surfactant is sodium lauryl sulfate (SLS) which can cause skin irritation (6). A good foam stability criterion is when subjected to a foam stability range of about 60-70% in the first 5 minutes. In each soap formula containing stearic acid so that the resulting foam stability is good enough to be proved in the foam stability data within the first 5 minutes.

3.2. Antioxidant Activity

Based on the analysis of variance, the addition papaya on solid soap, was no significantly affected to antioxidant activity. Antioxidant properties of sample soap caused by active substance from papaya fruit such as α -tocopherol, ascorbic acid, beta carotene, flavonoids, vitamin B1, and niacin (7). Polyphenols as antioxidants work through four mechanisms, such as damaging free radicals, preventing the formation of free radicals through hydrogen bonding, deactivate singlet oxygen which acts as a free radical in the body, and to be bonded with metals. We're using solvent methanol because in flavonoid raw materials and phenolic acids will dissolve in methanol and is recognized as the main phenolic fraction with antiradical activity (8). The The average of antioxidant activity and ability of sample soap to scavenge the DPPH radical measured as IC₅₀ will shown in table 4.

Table 4 Antioxidant activity on solid soap added papaya from different concentration

Formula	Antioxidant activity (%)					IC ₅₀ (ppm)
	500	1000	1500	2000	2500	
F I	13.41	16.01	15.88	17.18	17.44	20,434
F II	14.19	14.97	15.36	18.09	20.69	11,929
F III	15.62	18.22	18.09	20.17	26.15	8,098
F IV	15.36	15.75	16.27	17.05	21.47	13,657
F V	17.05	16.66	18.35	19.39	18.87	26,034
F VI	13.28	13.41	16.53	20.17	20.3	19,405

This suggests that the antioxidant activity in soap samples is very small and has significantly decreased antioxidant activity after formulated into soap. Based on research from the Journal International Food Research obtained IC₅₀ value of mature papaya fruit about 4300 ppm with 90.67% antioxidant activity (9).

3.3. Effectiveness in Cleaning

Dirt removal test by comparison of control positive soap (without enzymes) with control negative (with enzyme). The process of lifting the dirt is removal of impurities from the fabric fibers (substrate) which includes the interaction between surfactant, dirt, and fabric surface. Wavelength 270 nm was chosen based on the maximum absorbance result of the washing solution and according to the Journal International of Research in Pharmacy and Science Department of Quality Assurance, B.N College of Pharmacy papain solution has a maximum absorption at wavelengths of 270 - 280 nm (11).

Table 5 Differences of control soap negative and positive, measurement removal dirt with absorbance of wash water substrate

Negative control	Absorbance	Removal dirt (%)	Positive control	Absorbance	Removal dirt (%)
E ₂	0.1963	7	B ₂	0.1537	5
E ₄	0.2994	11	B ₄	0.194	7
E ₆	0.3108	11	B ₆	0.2061	7
E ₈	0.3509	13	B ₈	0.2252	8
E ₁₀	0.526	19	B ₁₀	0.2799	10
Absorbance dirt		2.8021			

Table 5 and 6 shown that negative soap control ability has higher dirt removal ability compared with positive control soap. So the rough papain enzyme contained in negative control soap has a role in removing more impurities in the washing process by the same treatment. But differences in soap removal ability of positive and negative controls are not significant. Because the use of the active substance rough enzyme papain is not a pure papain enzyme that is by tapping the sap from young papaya fruit. In crude papain enzyme there are still components of contaminants that interfere with the work of papain enzyme. According to the journal Biochem J. University of London papain is heavily contaminated by chymopapains A and B and there are other impurities that solution in crude papain enzyme during tapping of papaya fruit.

Table 6 Differences of control soap negative and positive, measurement removal dirt with mass of substrate

Negative control	Mass (gr)	Removal dirt (%)	Positive control	Mass (gr)	Removal dirt (%)
E ₂	1.2041	19	B ₂	1.3038	13
E ₄	1.1316	24	B ₄	1.2117	19
E ₆	1.0661	28	B ₆	1.0705	28
E ₈	1.0361	30	B ₈	1.0658	28
E ₁₀	1.0142	32	B ₁₀	1.0504	29
Mass of dirt substrate (gr)				1.4968	

4. Conclusion

Based the research it can be concluded that the addition of crude enzyme papain on the solid soap have affected to removal dirt higher than soap without enzyme, and did not significantly affected to the fatty acids, alkali free, pH, specific density and foam stability. The best formula based on the activity antioxidant which safe for skin and full meet criteria SNI 1996 was formula IV. Formula IV (addition 30% papaya fruit as antioxidant and 1.5% crude papain enzyme) had pH 10.35; value of fatty acid 79%; alkali free 0.108%; specific density 1.0595 gr/mL; and foam ability 78% after stand for 5 minutes.

5. Reference

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