Formulation of Natural Papaya Soap with Papain Enzyme

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***Abstract*— This research was done to formulate the natural papaya soap by adding papain enzyme and papaya pulp without any fragrance and to determine the concentration of vitamin c present in the sample of soaps. Nowadays, people are very concern about the ingredients of skincare especially cleanser and soap. The use of papain enzyme in the natural papaya soap is to prevent skin from irritation. The soap was produced using melt and pour soap, while papain enzyme and papaya pulp were added with different formulation. Then the vitamin C concentration in each formulated soap was determined using High Performance Liquid Chromatography (HPLC). The highest concentration of vitamin c (120.25 µg/mL) was found in sample formulated with 30% (w/v) papaya pulp and 1% (w/v) papain enzyme.**

Keywords*— *Ascorbic Acid, Papaya, Melt and pour soap, Natural soap, Papain enzyme and Vitamin C.

I. INTRODUCTION

Soaps are sodium and potassium salts or fatty acids derived from vegetable and animal oils [1]. The transesterification process is influenced by several process parameters such as reaction temperature, type of alcohol used and their oil to methanol molar ratio, reaction time and agitation speed [2]. The uncontrolled formation of free radical formation released to the air especially in the high urban area lead to negative effects on the air quality. This condition may cause human skin continuously contacts to those radicals in the air. 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 [3].

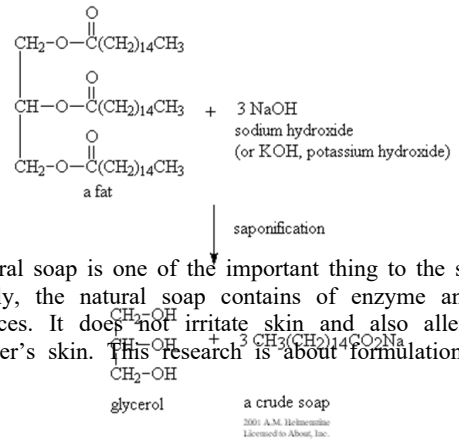

Figure 1: The chemical equation of the soap formation

Natural soap is one of the important thing to the skin barrier. Basically, the natural soap contains of enzyme and nontoxic substances. It does not irritate skin and also allergic to the consumer's skin. This research is about formulation of natural papaya soap by addition of papain enzyme (Walters, et. al 2012). Papain is a which found naturally in papaya (carica papaya) manufactured from the latex of raw papaya fruits. Papaya's soap has high activity in the breakdown of proteins into peptides and amino acids. Crude papain enzyme is developed into active ingredients of solid soap formulation with the antioxidant that come from fresh papaya to enhance the useful soap [5]. Antioxidants are the substances that the body needs including the skin to neutralize free radicals and also prevent damage skin that caused by free radicals.

Papain enzyme is also rich of vitamin (Özkan,et.al 2011). The function of the vitamin C is stimulating collagen synthesis and facilitate in antioxidant protection against UV-rays. High concentration of vitamin C in the skin means that it has a number of important biological functions that are relevant to skin health [7]. Vitamin C influences the collagen synthesis is by stimulation of lipid peroxidation. Collagen formation is carried out by the fibroblasts in the dermis on the skin. The generation of the base of the membrane and dermal collagen matrix will form [8]. Vitamin c is important to against both UV exposures such as UVB and UVA. Vitamin C not does not absorb UV light but exerts an UV protective effect by neutralizing free radicals while this effect is not seen with sunscreens. Under laboratory, it seems that 10% topical vitamin c can reduce UVB induced and sunburn cell formation 40-60% [7].

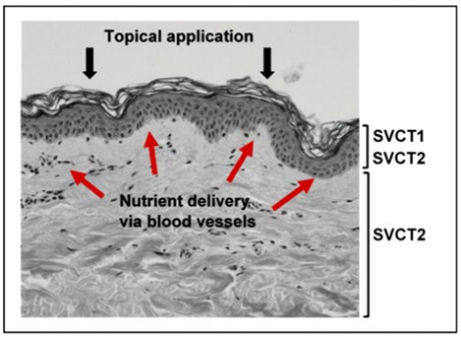

Figure 2: The path of the nutrients diffusing into the blood plasma

Vitamin C has a potential anti-inflammatory activity and can be used in conditions like acne vulgaris and rosacea. It can initiate wound healing and avoiding post-inflammatory hyperpigmentation [8]. Therefore, the rich of vitamin C in the papaya fruits can built new cell by remove dead skin and enhance the antioxidants protection to the skin.

As protection of the body from the outside, the skin has various problems such as dry skin, premature aging and chronic disease like skin cancer. These are the impacts from the air pollution resulting in many free radicals, exposure ultraviolet (UV rays) and also less care for cleanliness. The main treatment for maintaining skin health is to use soap regularly. By using of soap regularly, it leaves the skin smoothly and clearly. The dirt that attached to the skin will be removed clearly [9]. Moreover, skin is

a parts of body that very difficult to handle especially face because everyone has their own skin problem. Some of the people are allergic to the fragrance that contain in the soap or cleanse. Otherwise, the toxic chemicals in the solid soap also tend to the allergic skin problems. The reddish of the skin colour is one of the allergic symptoms that can be seen after using the soap [4]. It means that the ingredient of the soap or cleanse is not suitable with their skin barrier.

Method to formulate the natural soap from papain enzyme is by saponification process. Saponification is one of the process that used to produce solid soap. Papain enzyme is extracted from the latex from the papaya fruit containing high of antioxidant [10]. It can be purchase from the supplier in powder formed. The pure powder papain enzyme will be added to the soap solution. In the experiment, transparent melt and pour soap is used. The ability of melt and pour soap has the characteristic of either being isotropic but the defining characteristic of melt and pour soaps are their ability to be produced, cooled, melted, and re-cooled to accomplish the same bar of soap that was originally produced. The most advantage of the melt and pour soap is the base has already through saponification and the lye is not required. Moreover, melt and pour soap is quick to produce and it also hardens in several hour after melt it [1]. Soap is very sensitive towards pH value. Commonly, pH range of the soap is 9-10. If the pH is higher or more than pH range, the harshness of the soap also high. Neutralize the body's protective acid mantle by alkaline agent that acts as a barrier against to the bacteria and viruses. Healthy skin has a pH of 5.4 until 5 [11].

The fragrance of the soap comes from the papaya fruit without addition of other fragrances. Sometimes, sensitive skin is not suitable to the fragrance and it can cause allergic to the skin. Moreover, a few properties must be considered in order to manufacture the best and high quality of the soap. Nowadays the consumers are like to take care and concern about the side effects of the using of soap especially cleanse.

Papaya is a potent of nutrients and available throughout the year. The botanical name is *Carica Papaya* that rich of orange pulp. Papaya is a potent of nutrients and is available throughout the year [12]. The whole parts of the papaya give the lot of benefits to us such as fruits, peel, seed and pulp. It not only for nutritional benefits but it also can be as medical properties. The benefits of the papaya are high contents of vitamin A, Vitamin B and Vitamin C, Iron, Fibre and Magnesium. Moreover, it have proteolytic enzymes like papain and chymopapain which acts as antifungal and antibacterial properties [5]. Eczema and others disease likes allergic can be cure by the *Carica papaya*.

Carica Papaya contains an enzyme which is papain that present in the bark, fruits, latex and leaves of the tree. Papain is an important bioactive substances recovered from the latex of the green fruit [12]. It is herbaceous and polygamous growing from a single stem stance a crown of the large palmate-shaped leaves. Papaya has been much studied in pharmaceutical because of the medical properties in the parts of the fruit and tree. Papaya is suitable for one of the skin care's ingredients [13].

Enzyme in the soap formulation give the best quality of the soap because of their properties. Enzyme is the organic substances that low of risk to irritate consumers skin. Benefit of the papain enzyme in the soap formulation is high in antioxidant that give the skin smooth and leave the health skin. Furthermore, rich in natural Vitamin A, Vitamin C and Vitamin E in the soap that come from the all the of the papaya fruit [10]. The main part that contribute the benefits to the skin is papaya (*Carica papaya*) latex also known as papain enzyme.

II. METHODOLOGY

Soaps making is very versatile because its broad in demand and Melt and Pour Soap is the method in formulation of the natural soap [14].

A. Materials

Melt and pour soap (transparent soap), papain enzyme in powder form is purchased from Green Global, pulp of papaya juice, ascorbic acid is purchased from MERCK, methanol, ethanol and deionised water.

B. Soap Preparation

Six samples of melt and pour soaps were prepared before adding the papain enzyme. The soaps were melted using double boiler at around 65-70 °C without stirring it. When the soaps were already melt, pour the papain enzyme with different concentration for each soaps sample. Then, the pulp of papaya juice is adding into the soap samples. The soap must keep stirring to make sure the soap was homogenized with papain enzyme and papaya pulp. Spray alcohol solvent (ethanol) onto the base surface to eliminate the air bubbles. Put the liquid soap into the mould to shape the soap. Let the liquid soap base cool for 24 hours at room temperature around 25 °C. Then, take out the solid soap from the moulding [15]. The samples of soap are ready to analyse.

Table 1 shows the amount of the papaya pulp and papain enzyme for each sample.

Table 1: Formulation of natural papaya soap samples

Samples	Amount of papaya pulp (%w/v)	Amount of papain enzyme powder (%w/v)
1	15	0.5
2	15	1
3	25	0.5
4	25	1
5	30	0.5
6	30	1

C. Standard solution preparation

An amount of 10 mg of Ascorbic acid were weighed accurately. Ascorbic acid is transferred into the 100ml of volumetric flask that contain 100mL of Methanol. The standard solution that have been prepared is 100ppm. Inject (value) the standard solution into the nozzle of HPLC system. The highest peak of the wavelength is recorded.

E. Sample preparation

An amount of 0.2g of the samples were weighed. Then, transferred it into 50mL volumetric flask contains 30mL methanol and sonicated the solution for 20 min at (temperature) in Ultrasonic Water Bath. The solution is injected into the nozzle of HPLC system.

F. HPLC – UV Method

Separation for qualitative analysis of the ascorbic acid are performed by HPLC – UV system (Perkin Elmer, UK) with diode array detected (operated at 278nm of its wavelength) and 4 µL sample loop. The binary mobile phase with (methanol-water (70:30, v/v). methanol as a component A while deionized water as component B. the flow rate of the injection sample and standard is 1.0 mL/min. Data are integrated VP series software and results are obtained by comparison with standards.

G. pH Determination

10 % soap solution is prepared with distilled water to determine pH using pH meter (Toledo). pH probe is inserted into solution after calibration with buffer solution (pH 4 and pH 10). The pH reading is recorded.

III. RESULTS AND DISCUSSION

A. Analysis of Vitamin C using HPLC

The wavelength used in the HPLC system is 278 nm and reverse phase HPLC on a C18 column [16]. HPLC is a binary mobile phase containing deionized water and methanol (70:30, v/v). Five standard solution of ascorbic acid are prepared with different concentration such as 100, 110, 120, 130 and 140 µg/mL. The area is varied due to the different concentration of the standard solution. The ascorbic acid is diluted with polar organic solvent which is 95% methanol.

From the variation of standard solution concentration, a calibration curve is plotted. The regression line that must be used to calculate the concentration of the vitamin C in different six samples.

(general equation)

$$y = 1895.5x - 160766 \quad \text{(Eq. 1)}$$

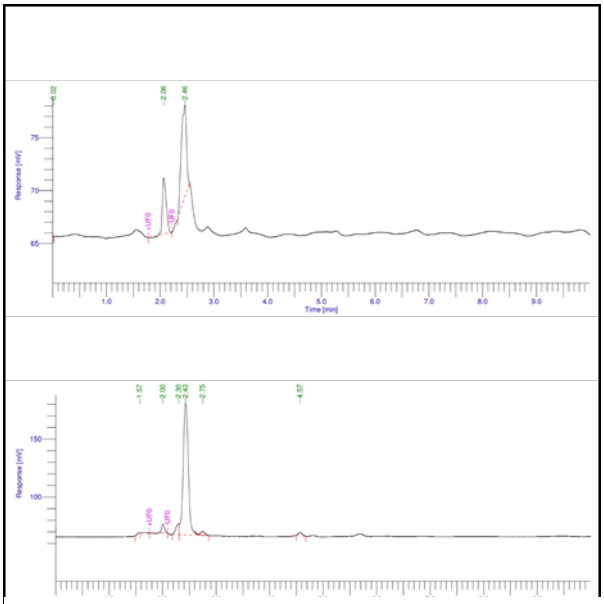

Figure 3: Representative HPLC chromatography of (a) standard ascorbic acid and (b) sample 1 at 278nm

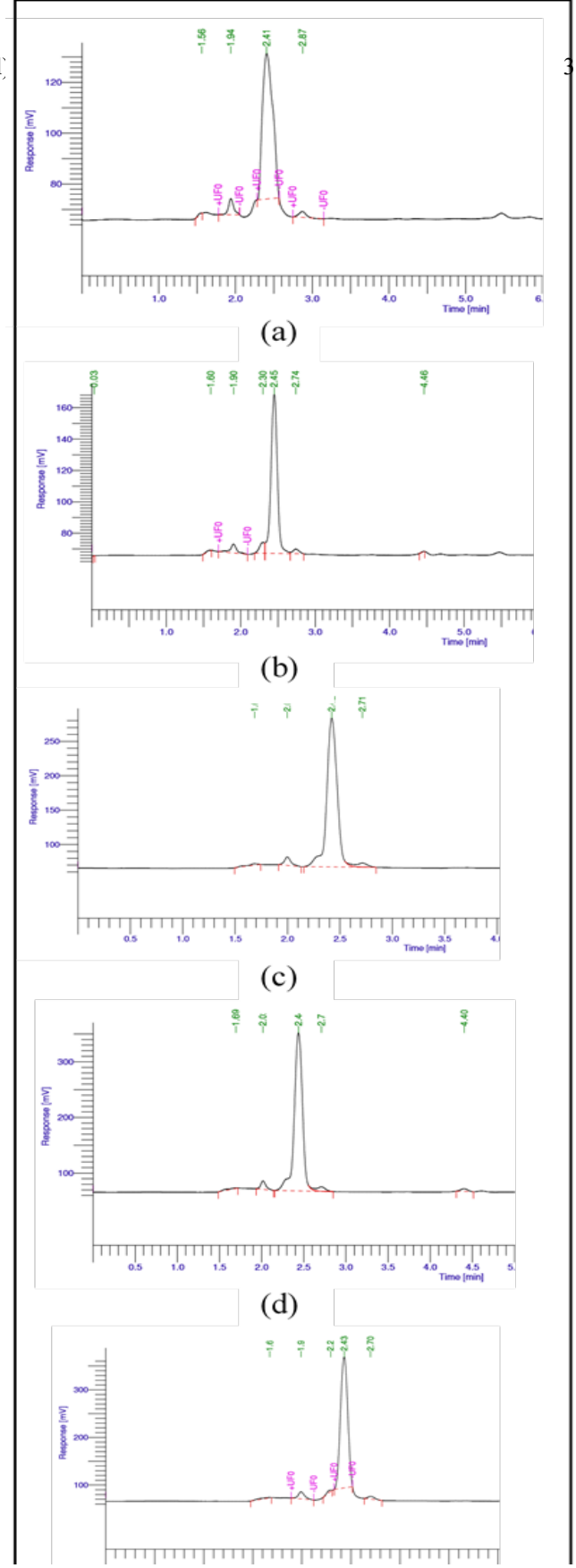

Figure 4: Response curves from the analysis of vitamin c using HPLC (a) sample 1 of papaya soap (b) sample 3 of papaya soap (c) sample 4 of papaya soap (d) sample 5 of papaya soap and (e) sample 6 of papaya soap at 278nm wavelength

Table 2: Concentration of vitamin c in each samples

Sample	Concentration of vitamin (µg/mL)
1	100.27
2	101.16
3	102.56
4	113.41

B. Determination of vitamin c in samples

5	118.69
6	120.25

Based on the regression equation (Eq.2), the peak selected for the standard solution at 1.938min. The relevant peak of the standard solution of ascorbic acid at 278nm is 2.065 min. The peak of the sample 1 must be around at 2.065 min. the vitamin c concentration in the sample 1 is 100.27 µg/mL.

The amount of the papaya pulp in the sample 1 is 15 %w/w of the soap while the papain enzyme is 0.5 %w/w. Furthermore, sample 1 is the least amount of papaya pulp and papain enzyme. The amount of the papaya pulp and papain enzyme different from the other samples. Therefore, the concentration of the sample 1 also different from others. Sample 1 is the least of the vitamin c concentration among other samples.

Peak for the sample 2 at 2.003 min. the range of the concentration vitamin c in this sample 2 is 101.16 µg/mL. The concentration of the sample 2 is greater than sample 1. It is due to the amount of papain enzyme and papaya pulp in each sample soaps. The amount of papaya pulp for the sample 2 is same goes to sample 1 which is 15 %w/w while the papain enzyme in the sample to is higher than sample 1 which is 1 %w/w. Papain enzyme also contribute vitamin c concentration to the sample.

At 1.913 min, the maximum peak for the sample 3 is seen. The concentration of the sample 3 is higher compared to sample 1 and sample 2. It is because the amount of the papaya pulp is more than sample 2 and sample 1 which is 25 %w/w while the amount of papain enzyme is same goes to the sample 1 which is 0.5 %w/w. The concentration is affected by the amount of papaya pulp and papain enzyme in the soap. The value for concentration of vitamin c is 102.56 µg/mL.

The concentration of the sample 4 is 113.41 µg/mL. at 1.997 min. the amount of papaya pulp for sample 4 and 3 is same while the amount of papain enzyme for sample 4 is more than sample 3 which is 1%w/w. However, the amount of papain enzyme for sample 4 is equal to the sample 2 but the concentration and area for sample 4 is higher than sample 2. It is because the amount papaya pulp for the sample 2 is less than sample 4 which is 15%w/w. Therefore, the concentration of the sample 4 is higher than sample 3.

At 2.015 min, the highest peak for the sample 5 is seen. The concentration of vitamin c in the sample 5 is 118.69 µg/mL. 30 %w/w of papaya pulp in the sample 5 is equal to the sample 6 but it more than others sample. The concentration must be greater than others samples except sample 6. It is proved that when the concentration of sample 5 is more than sample 1,2,3 and 4. The amount of the papaya pulp is affected the concentration of vitamin c in the sample. In fact, that papaya pulp is contains high vitamin c and vitamin e in its fruit. Actually, all part of the papaya fruit is containing the high vitamins that give more benefits to the user and consumers. One of the advantages of the vitamins in the papaya fruit is it can build new cell by remove dead skin and enhance the antioxidants protection to the skin [17].

For the sample 6, the amount of the papaya pulp is 30 %w/w is same to the sample 5. But the different of sample 6 with sample 5 is the quantity of the papain enzyme. The amount of the papain enzyme in the sample 6 is 1 %w/w while sample 5 contain 0.5 %w/w.

The concentration of the sample 6 must be more than sample 5 which is 120.25 µg/mL. When the area increase, the concentration of vitamin c also increases. Based on the statement above, the papain enzyme is more nutrient that can be found in the leaves, bark, latex and also fruit [18]. Papain enzyme also develop into the antioxidants that come from fresh papaya fruit [5]. Antioxidant is important toward to the skin as a barrier to remove free radicals that naturally in the skin and as a prevention to the free radicals at the surrounding from attach to the skin. It also can be considered as a potential to contribute the concentration of the vitamin c in the sample.

In overall, the amount of papaya pulp and papain enzyme effected the concentration of vitamin c and the area from the injection of the sample in HPLC-UV. If the amount of the papaya pulp, increase of the concentration of vitamin c. meanwhile, the amount papain enzyme increase so the concentration of vitamin c also increases. In fact, f the many researchers have reported a direct relationship between total phenolic content and antioxidant activity in numerous seeds, fruits and vegetables. Basically, papaya pulp and papain enzyme have a high amount of vitamin c in the papaya fruit and also its enzyme. High of the vitamin c in the sample is better because vitamin c has more benefit to the skin. Generally, skincare that contain high concentration of vitamin c is the most suitable for the skin and give the positive impact to the skin of consumers.

In conclusion, all the parts of a fruit and all of fruits have not equal amounts of edible part. From the previous study, the concentration of the vitamin c in the *Phyllanthus Emblica* is 71.62 µg/mL [16]. Meanwhile, papaya have high amount of vitamin c. therefore, the amount of vitamin c in the best sample 6 is higher than Phyllanthus Emblica which is 120.25 µg/mL.

C. Determination of pH

Samples	pH
1	9.12
2	9.11
3	9.11
4	9.11
5	9.12
6	9.11

The pH of the soaps is nearest to each other. Six sample of soaps is made from the soap base which is melt and pour soap. Therefore, the pH of the soap is slightly same. The addition of papaya pulp and papain enzyme in the soap does not changed the pH of the soap [14]. All the pH soaps are in range which is 9-10. The soap must be in the alkaline medium to neutralize the skin pH.

IV. CONCLUSION

From the analysis that have been done, the best sample by determining of concentration of vitamin c is sample 6. It means that, sample soap 6 have high vitamin c which is 120.25 µg/ml that can give more benefit to the skin. The pH of the sample 6 also has an optimum pH that can be applied to all type of skin especially sensitive skin. Skincare that contains high vitamin c is the best product to use.

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