

Improvement in extraction and sensory properties of soapnut extract by fermentation

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Sapindus saponins are potential biosurfactants that can widely be used to replace many chemical cleaning products. This study aimed to investigate the water extraction of saponins from the pericarps of *Sapindus mukorossi* and enhance the sensory properties of the extract by yeast fermentation. Extraction conditions including temperature, solid-to-liquid ratio, extraction time, and number of extraction times were studied. A yield of 21.4% was obtained by 2 h of extraction at 80 °C with a solid-to-liquid of 1/6 (w/v) and two times. Fermentation was used to purify the *Sapindus* extract, inoculum amount and fermentation time were optimized. The fermentation by *S. cerevisiae* (2%) within 4 days significantly improved the color and smell of aqueous extract, turbidity decreased by 75.6%, total sugar content decreased by nearly 50% and saponins content slightly decreased. These results could contribute to the development of industrial-scale production of *Sapindus* saponins.

Keywords: Saponin, *Sapindus mukorossi*, fermentation, *Saccharomyces cerevisiae*.

INTRODUCTION

Sapindus mukorossi Gaertn (also known as soapnut) is a deciduous tree that is widely distributed in Asia's tropical and sub-tropical regions^{1,2}. Soapnut is tolerant to reasonably poor soil and each tree can produce 30–35 kg of fruit per year³. The major active components of its pericarp are saponins, which have been confirmed to be effective natural nonionic surfactants and have very low toxicity, hemolysis, and skin irritability⁴. *Sapindus* saponins have also been proven to possess many pharmacological effects, including anti-microbial, anti-fungal, anti-dermatophytic, and anti-inflammatory activities⁵. Therefore, having great potential in developing functional cosmetics and daily cleaning products^{6,7}. The natural surfactants were also proven to be readily biodegradable and have excellent performance for environmental remediation, as opposed to chemical and synthetic surfactants⁸. As a result, *Sapindus* saponins which are environmentally friendly surface-active agents, have raised great interest worldwide in terms of the replacement for chemical cleaning products.

Sapindus saponins can be extracted efficiently by water due to their compatibility; hence water – an inexpensive and safe solvent has been investigated for *Sapindus* saponins extraction in many research⁹. However, the *Sapindus* water extract (SW) has some undesired sensory characteristics such as color, odor, and turbidity. The dark brown color, intensely sour odor, and higher turbidity during the storage time of the SW have limited its application in commercial cleaning products¹⁰. A method to decolorize the SW by hydrogen peroxide has been investigated in some research¹¹. Despite its quick efficiency, the strong oxidizer partially affects the surface activities of the SW and raises safety concerns in daily uses.

Sapindus crude extract contains an abundance of carbohydrates, a few proteins, calcium, magnesium, iron, and

other impurities¹², which correlates with the undesired sensory properties of the SW^{13,14}. Enzymatic treatment and bacterial fermentation are effective methods in purifying fruit/plant extract, improving its quality and sensory characteristics^{15,16}. The enzymatic process is quick, productive, and targeted for certain substances, while the fermentation method is less expensive, resulted reducing various components and producing some by-products^{17,18}. Microbial fermentation to purify the *Sapindus* saponins after water extraction was a recently introduced method¹⁹. After purification, *Sapindus* fermentation product (SWF) has been shown to enhance various properties, such as surface activities, microorganism inhibition, and anti-acne^{9,19,20}. The fermentation process also showed significant positive changes in sensory characteristics (color, odor, and turbidity)^{21,22}. Via the activities of bacteria, the dark brown color of the SW has been transformed into light yellow-brown, reducing the turbidity and creating a better smell. Therefore, fermentation is grasping attention as a potential method of purifying *Sapindus* saponins extract.

Traditional fermentation of *Sapindus* extract is also applied by many people to obtain the SWF as a multi-purpose cleaner²³. This method is inexpensive and straightforward, whereas spontaneous and uncontrolled fermentation has created a real challenge in achieving consistent quality of the final products²⁴. Therefore, some issues regarding standards and efficiency should be carefully considered, primarily to conduct on a large scale. This study aimed to optimize the water extraction of *Sapindus* saponins, which could be used to develop more extensive manufacturing processes. Then yeast fermentation is employed to remove impurities and improve the sensory properties of *Sapindus* extract, and natural fermentation was also compared.

EXPERIMENTAL DESIGN

Materials

Samples of *S. mukorossi* pericarp were harvested in Tay Ninh province, Vietnam (in January); *Saccharomyces cerevisiae* dry yeast was purchased from Brenntag Vietnam Co. Ltd; Analytical standard Oleanolic acid, D-glucose were purchased from Sigma Co. Ltd (USA). Besides, other chemicals such as Diatomite (90%), Vanillin (99%), and Sulfuric acid (98%) were purchased in Xilong, China.

Extraction of *Sapindus* saponins

For large-scale production, pericarps of *S. mukorossi* were kept at their initial size. *Sapindus* pericarps were extracted with distilled water by maceration and stirring under specific conditions. The first extract was crudely filtered through a gauze and the residue obtained was continuously extracted with the same procedure. Subsequently, the product solutions were combined and thoroughly mixed to obtain the *Sapindus* saponins extract (SW) for the fermentation process or filtered with filter aid powder (diatomite) for further analysis. The extraction conditions such as temperature, material-to-liquid ratio, and extraction time were investigated.

Yeast fermentation of *Sapindus* saponins extract

Yeast Activation

Before inoculation, 3 g of the *S. cerevisiae* dry yeast was rehydrated in 15 mL of distilled water and 15 mL of SW. The yeast solutions were activated for 30 min in a water bath at 35 °C and used for further fermentation.

Fermentation Method

The activated yeast was inoculated into 150 mL of SW obtained from the extraction process. The containers were sealed and incubated at room temperature (25–35 °C), away from direct sunlight. Then, the fermented extract was then autoclaved and filtered with filter aid powder to obtain the *Sapindus* fermentation product (SWF). Fermentation parameters such as time and inoculum amount were investigated.

Traditional fermentation method

Sapindus pericarps were pre-washed in tap water to remove dust and impurities. Then *Sapindus* pericarps, jaggery, and water with the ratio of 1:1:10 (w/w/w) were added together and thoroughly mixed in a container with a lid. Subsequently, the immersion was covered, placed at sunny places, and fermented in anaerobic conditions. At the beginning of the fermentation process, the mixture should be gently stirred once a day to provide oxygen, boosting the growth of microorganisms. Until a thin layer of microorganisms appeared on top, the immersion can completely be sealed and fermented in anaerobic conditions for three months. The sample solutions were collected weekly and filtered with filter aid powder to determine the total saponin content.

Determination of total saponins

The total saponins of SW and SWF were determined according to the vanillin-sulfuric acid assay²⁵. Oleanolic acid was used as the saponin standard.

Sample measurement: 0.3 mL of vanillin 8% solution and 3 mL of sulfuric acid 72% were respectively added into 0.3 mL of sample diluted several times. The solutions were evenly mixed using a vortex shaker, warmed in a water bath at 60 °C for 10 min then cooled in ice-cold water for 10 min. The absorbance was determined at 538 nm by using a UV-Vis spectrophotometer (model GENESYS™ 10S, ThermoFisher Scientific Inc., USA).

$$Y(\%) = \frac{W_s}{W_p} \times 100\% \quad (1)$$

Where: Y_i (%) is the extraction yield of total saponins
 W_s (g) is the weight of total saponins in the extract.
 W_p (g) is the weight of the *Sapindus* pericarp.

Determination of total sugars

The total sugars were determined according to the phenol – acid sulfuric²⁶. The absorbance was measured at 490 nm, D-glucose was used as the sugar standard. The quantitative colorimetric method can determine the content of sugars and their methyl derivatives, oligosaccharides, and polysaccharides.

Sample measurement: 0.5 mL of phenol 5% solution and 2.5 mL of concentrated sulfuric acid were respectively added into 0.5 mL of sample diluted several times. The solutions were allowed to stand for 10 min, then they were evenly mixed and placed for 20 min. The absorbance was determined at 490 nm by using a UV-Vis spectrophotometer (model GENESYS™ 10S, ThermoFisher Scientific Inc., USA) and the concentration of total sugar (mg Glu/mL) was calculated.

Evaluation of sensory properties

Evaluation of turbidity

The turbidity was evaluated by UV-Vis spectrophotometer (model GENESYS™ 10S, ThermoFisher Scientific Inc., USA) according to Batch's report²⁶. Principle of the evaluation was based on the amount of light absorbed, scattered, or reflected by particles in the solution. The absorbance was determined at 860 nm.

$$OD_{860} = OD_S - OD_B \quad (2)$$

Where:

OD_{860} indicated the turbidity of *Sapindus* solution at 860 nm

OD_S is the absorbance of a sample

OD_B is the absorbance of a sample filtered by 0.2 μ m microfilter.

Evaluation of color

The sample colors were measured by a CR-400 Chroma Meter. The L axis describes the Lightness of the color, going from absolute black (L = 0) to absolute white (L = 100). Samples were measured color directly without any dilution in a 1 cm glass cuvette.

Evaluation of odor

The odor evaluation focused on odor quality and preference. A group of 15 people was invited to evaluate the odor of the samples measured on a 5 – point scale. The samples were presented in random order and marked by each attendant (attendants were required to smell water before starting a new sample). The data were statistical analyses by ANOVA.

The 5 – point scale described for:

- 1 – very unpleasant
- 2 – unpleasant
- 3 – moderate
- 4 – pleasant
- 5 – very pleasant.

Analysis data

The procedure was repeated three times for each sample. The mean and standard deviation of the results were calculated using Microsoft Excel program (Microsoft Inc., Redmond, WA, USA). Experiment data were analyzed using a one-way analysis of variance (ANOVA) test in the SPSS program (IBM Company, USA) with a level of significance at 5%.

RESULTS AND DISCUSSION

Effects of extraction conditions

Sapindus saponins can be extracted efficiently by water and ethanol. Although the yield of total saponins with

ethanol extraction was slightly higher, water was preferred in *Sapindus* saponins extraction because it is an inexpensive and safe solvent^{9,12}. Since *Sapindus* saponins were stable with high temperature^{1,27}, it was evident that increasing the extraction temperature could improve the efficiency and reduce extraction time without leading to degradation of the compounds. However, extraction of *Sapindus* saponins was generally conducted at low temperatures (40–60 °C)¹². It is because water extraction under high temperatures could increase the swelling ability of starch Inoculation amount-based components²⁸ that fully existed in the *Sapindus* extract, making the filter process impossible. In this study, *Sapindus* pericarps were kept at their original size, hence the swelling of the material was minimized, and the water extraction of *Sapindus* saponins has become more possible with higher temperatures. As can be seen, this slight adjustment has made the extraction process of *Sapindus* saponins more suitable and efficient on a larger scale.

Factors such as temperature, material-to-solvent ratio, extraction time, and the number of times of extraction were considered to have a significant effect on the extraction yield. The results of the single-factor test are shown in Figure 1. An extraction temperature of 80 °C, a solid-to-liquid ratio of 1/6 (g/mL), an extraction time of 120 min, and extraction two times gave the best results. Under these conditions, the water extraction of saponins yielded 21.4%.

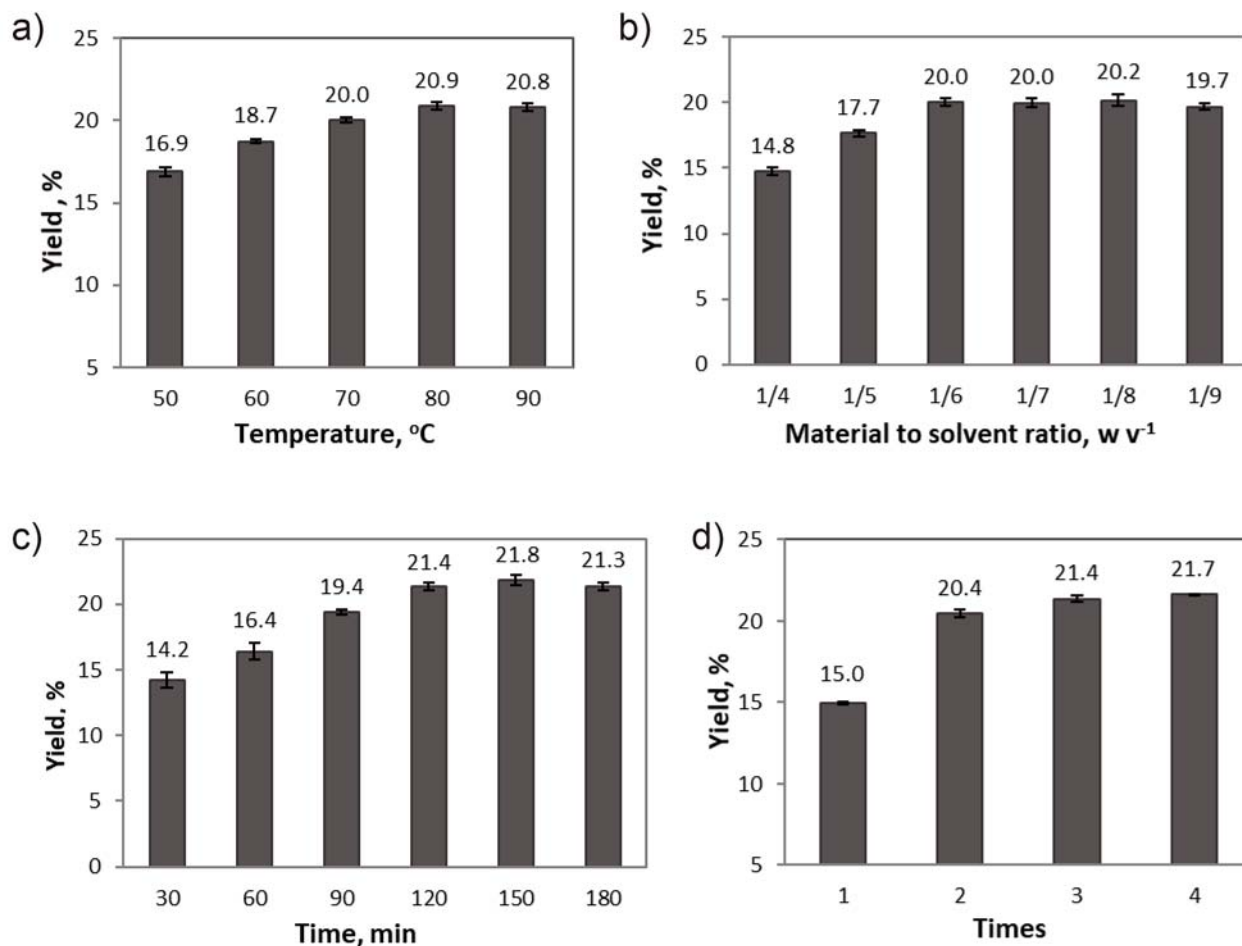


Figure 1. Effect of extraction conditions (temperature (a); material to solvent ratio (b); time (c) and times (d)) on the yield of total *Sapindus* saponins

Fermentation condition

To improve the purity as well as sensory properties of SW, further fermentation by *S. cerevisiae* was investigated. Since the optimal temperature for the growth of *S. cerevisiae* was 28–32 °C, the fermentation was conducted at room temperature, and factors such as fermentation time and inoculation amount were optimized.

Fermentation time

Each container was filled with 150 mL SW inoculated with 1.5% (v/v) activated yeast and incubated at room temperature for 1–7 days. The concentration of total sugar decreased markedly, and saponins were also consumed in small amounts during the fermentation process due to the growth of yeasts (Fig. 2). At the beginning of 4 days, the total sugar content reduced rapidly from 42.86 ± 1.58 mg Glu/mL to 23.04 ± 1.81 mg Glu/mL and then became quite stable. In contrast, the concentration of saponins declined significantly after 4 days of fermentation. The potential explanation was the preference of yeast in using carbohydrates and proteins in the *Sapindus* extract; hence the amount of sugar in the first four days decreased tremendously, and the saponins content was less affected. Some previous studies also show that *Sapindus*'s fermentation time of 4 days is necessary. At this time, the total sugar content dropped to the lowest level, while the purity of saponin and ethanol content increased significantly^{9, 12}.

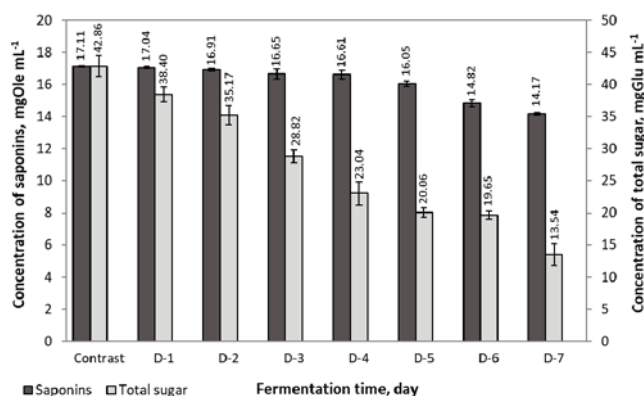


Figure 2. Effects of fermentation time on saponins and total sugar content

In terms of sensory characteristics, SWF also showed a vast improvement in turbidity, color, and smell after 4–5 days of fermentation, which will be presented afterward. Consequently, the optimal fermentation time of SW by *S. cerevisiae* was 4 days.

Inoculation amount

As can be seen clearly, the inoculation amount showed little effect on saponins but significantly affected the sugar content (Fig. 3). The concentration of saponins only declined less than 1 mg Ole/mL when the inoculum amount changed from 0.5% to 3%. There was a significant drop in total sugar content when increasing the inoculum amount to 2% (from 39.66 ± 0.60 mg Glu/mL to 21.74 ± 0.93 mg Glu/mL). The increased inoculum volume (3%) is related to a change in the concentration of total sugar. When considering sensory properties, the SWF also showed evident progress with 2% or 2.5% of

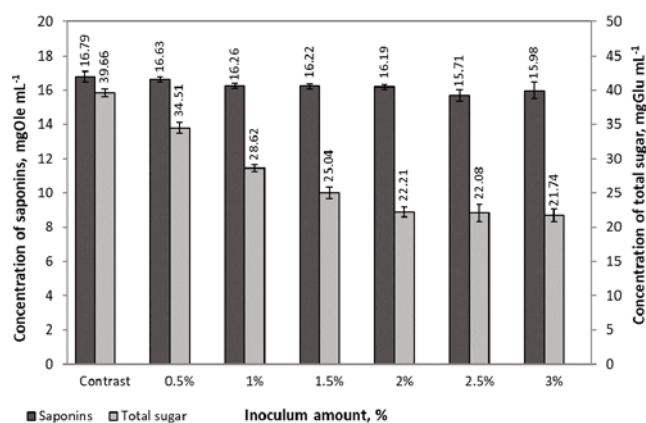


Figure 3. Effects of inoculum amount on saponins and total sugar content

the inoculum amount. Therefore, it was concluded that the appropriate amount of inoculum was 2%.

Effects of fermentation on sensory characteristics of *Sapindus* extract

The dark brown, intensely sour odor, and high turbidity of the *Sapindus* extract have limited its application in commercial cleaning products. By purifying the crude extract, fermentation has made significant improvements in the sensory properties of the final product.

Turbidity and color

The SW treated by fermentation showed a remarkable decrease in turbidity over the days (Fig. 4). The decreasing stage of turbidity was most apparent in the

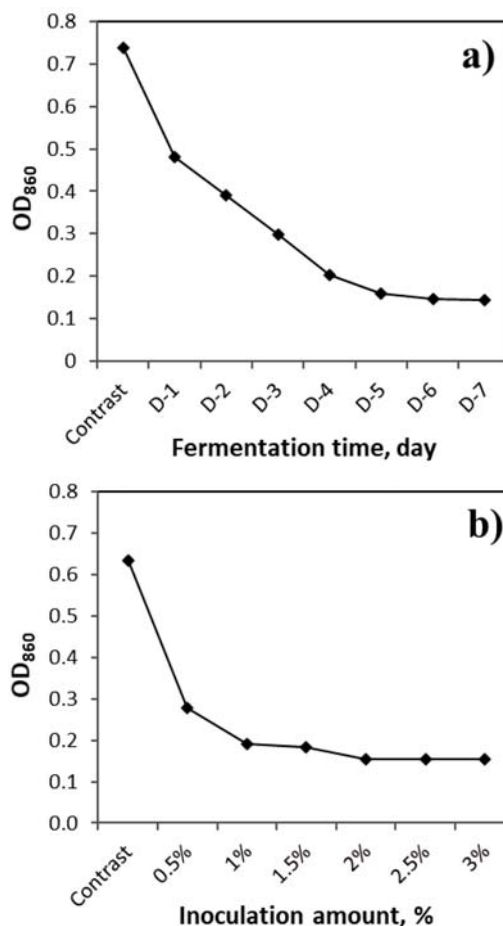


Figure 4. Effects of fermentation time (a) and Inoculation amount (b) on turbidity of *Sapindus* extract.

first 4–5 days of fermentation, and with the inoculation amount above 1% (improved more than 70%). In terms of color, the SWF showed an increase in the lightness axis (Table 1) and fermentation had changed the dark brown of the SW into a light yellow-brown color (Fig. 5). The improvements in turbidity and color have made the final product's appearance possible to apply in commercial cleansers.

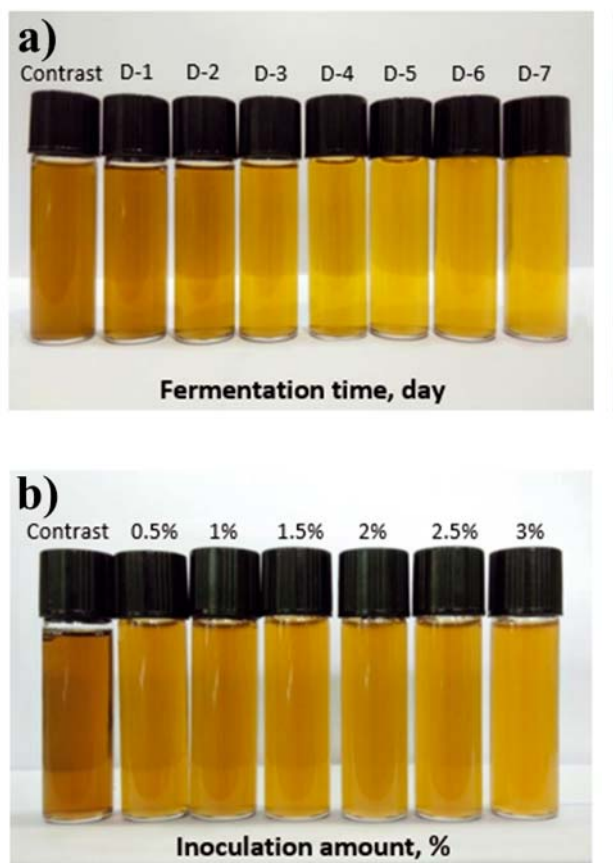


Figure 5. Effects of fermentation time (a) and Inoculation amount (b) on color of *Sapindus* extract

The activities of *S.cerevisiae* can explain the improvement in turbidity and color of the SW. The cloudiness of *Sapindus* crude extract was due to an abundant amount of impurities such as sugars, proteins, and polysaccharides¹⁹ which were consumed by yeast. Additionally, fermentations helped to reduce a large number of sugars that could be responsible for the browning intensity due to caramelization and participation in Maillard reactions during a long time of extraction¹³.

Odor

The *Sapindus* extract was intensely sour in odor, which might create displeasing experiences for customers. However, after being treated by yeast fermentation, the products showed great changes in aroma, which was more familiar and tolerant to most people. The odor

of samples was evaluated on a 5 – point scale based on odor quality and preferences.

Table 2. Sensory ratings of *Sapindus* extract before and after fermentation by *S. cerevisiae*

Fermentation time			
Contrast	D-3	D-4	D-5
1.93±0.7 ^a	2.93±0.8 ^b	3.53±0.9 ^{c2c}	3.6±0.51 ^c
Inoculum amount			
Contrast	1.5 %	2 %	2.5 %
1.87±0.74 ^a	2.73±0.46 ^b	3.27±0.8 ^b	3.27±0.88 ^b

The results showed that the aroma of *Sapindus* extract after being fermented was given a higher mark, especially after 4–5 days of fermentation and the inoculum amount of 2% or 2.5%. (Table 2). The changes in the odor of SWF can be explained by various volatile compounds produced by *S. cerevisiae* during a fermentation process. This yeast strain was known to produce significant amounts of alcohols, ethyl, acetate esters, and ethyl esters, which were generally associated with fresh fruit and citrus aromas²⁹. Also, the fermented products by *S. cerevisiae* were characterized as having aromas of fruity apple, bakeries, yeast, dairy, and acidity³⁰, which was preferable to most experiencers.

Natural fermentation of *Sapindus* saponin

Traditional fermentation is applied by many Vietnamese people to obtain the SWF as a dish- or cloth-washing liquid. Although this method is relatively affordable and simple to perform, it is considered time-consuming and produces low-quality products (Fig. 6).



Figure 6. *Sapindus* solution conducted by traditional fermentation after 1, 5, 10 weeks (W-1; W-5 and W-10, respectively)

The extraction efficiency of *Sapindus* saponins by natural fermentation was illustrated in Figure 7. The extraction process of saponins by natural fermentation was much more than heat and stirring-assisted extraction (yielded 21.4% in 2 hours). After the first week, the extraction yield was only 12.7% and gradually increased to the peak of 20.1% after 5–6 weeks. However, the weight of saponins extracted decreased significantly after 8 weeks of fermentation. The explanation for this tendency is the

Table 1. Effects of fermentation on the lightness of *Sapindus* extract

		Fermentation time						
	Contrast	D-1	D-2	D-3	D-4	D-5	D-6	D-7
The L axis	27.32	28.73	29.03	29.03	29.56	29.41	29.46	29.51
		Inoculum amount						
	Contrast	0.5%	1%	1.5%	2%	2.5%	3%	
The L axis	26.68	27.24	27.66	27.69	28.90	28.73	28.60	

activities of microorganisms during the natural fermentation process. After the maximum amount of *Sapindus* saponins has been obtained, the microorganisms continue to grow and spend the extracted saponins, leading to a gradual decrease inefficiency. Generally, the natural fermentation process was finished after 3 months to collect the SWF. Therefore, the low content of saponins in *Sapindus* solution was unavoidable³¹.

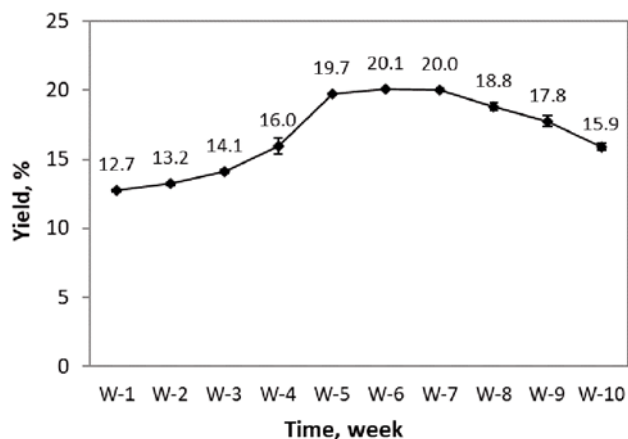


Figure 7. Extraction yield of *Sapindus* saponins by natural fermentation

Besides, the natural fermentation of *Sapindus* extract is spontaneous and uncontrolled. Consequently, achieving consistent quality in the final product is the major challenge³². Naturally fermented products come with a diverse bacterial community and contamination of unwanted which might fail to meet the quality assurance and raise many customer concerns. Therefore, the naturally fermented product of *Sapindus* extract is not suitable to become a commercial product. In the quest for better quality and process management, yeast fermentation after water extraction is a promising approach to industrial scale.

CONCLUSION

In this study, water extraction and yeast fermentation were optimized to purify the SW and enhance its sensory characteristics, traditional fermentation was also examined. Under 2 hours of extraction at 80 °C with a solid-to-liquid of 1/6 (w/v) and 2 times, the yield of *Sapindus* saponins reached 21.4%. Fermentation by *S. cerevisiae* with inoculate amount of 2% within 4 days was the ideal condition to purify *Sapindus* extract. While the saponins concentration of SWF slightly decreased, the total sugar content reduced approximately by 50%, turbidity decreased by 75.6% and obvious improvements in color and odor were observed.

In conclusion, yeast fermentation was a potential method to purify and improve the poorness in sensory properties of the crude *Sapindus* water extract (the fermentation had changed the dark brown of the SW into light yellow-brown color and sensory ratings from 1.93 ± 0.7 and 3.6 ± 0.51 before and after fermentation by *S. cerevisiae*). These results could contribute to the development of large-scale production of *Sapindus* saponins and their applications in commercial products.

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