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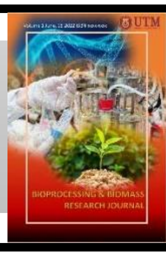

Research Article

Microwave Drying of Sacha Inchi (*Plukenetia Volubilis* L.) Seed for Improving Oil Yield Extract

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ABSTRACT

Sacha inchi oil became a popular edible oil due to its fatty acid profile. Due to the good fatty acid nutrient, sacha inchi was chosen to be investigated to get the high oil yield by maintaining the quality of the oil. The purpose of this study was to investigate the effect of drying power and drying time on the oil yield extracted using aqueous enzymatic extraction. Besides, the extracted oil colour and total phenolic content at different drying power and drying time also were determined. The fatty acid of the highest oil yield was investigated. The drying method used was microwave oven drying while the extraction method used was aqueous enzymatic extraction. The total phenolic content was analyzed using the Folin-ciocalteu reagent while the analysis of fatty acid was conducted using High-Performance Liquid Chromatography (HPLC). The result showed that drying power and drying time during drying process have affected the moisture content of the seed which later influenced the yield of sacha inchi oil. The oil yield that extracted from the set of seed dried at medium drying power is the highest. The total phenolic compound increased with the rising of the drying power and drying time, but the results might be affected by another chemical compound, 5-hydroxymethylfurfural in the oil. However, the highest sacha inchi oil yield resulting negative value of fatty acid. To obtain a better yield, de-emulsification was recommended to be include in the extraction method. The other factors which are particle size and concentration of the enzymes also need to be considered.
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INTRODUCTION

Plukenetia volubilis L. or sacha inchi come from Euphorbiaceae family and mainly found grown in the tropical rainforest of the Amazon region of South America. Sacha inchi oil contains the lowest saturated fatty acids (6.8%) compared to olive oil (14.2%), rapeseed oil (7.5%), and linseed oil (9.4%) (reference). There are two types of unsaturated fatty acids: polyunsaturated (PUFA) and monounsaturated (MUFA). The difference between MUFA and PUFA is the number of double bonds: PUFA contains more than one carbon-carbon double bond; MUFA contains only one carbon-carbon double bond. Olive oil, one of the popular healthy oils in the market, was found to have 75.0% of monounsaturated fatty acid which was much more than sacha inchi oil, 8.6%. Yet, the polyunsaturated fatty acid content in sacha inchi oil, 84.6% including both omega-3 and 6 was much more than olive oil, 10.8% (Kodahl, 2020). The risk of having Alzheimer's disease could be reduced by consuming high omega-3 fatty acids in a daily diet. It was due to omega-3 fatty acids that could improve memory performance (Moyad et al., 2005). Based on the research of Aryal et al. (2019), the phenolic compounds could undergo the free radical exhibition, having metal resistance and preventing oxidative stress which strongly clarified that the phenolic compound was playing a significant role in

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antioxidant activity. Both Goyal et al. (2002) and Chirinos et al. (2013) found the value of total phenols in the seed between 64.6 to 80.0 mg GAE/100g seed. While the total phenolic compound (TPC) in oil was lower than the seed (6.20 mg GAE/100g oil).

Microwave heating is a type of electromagnetic heating that works by using high-frequency electromagnetic waves to generate heat in a material (Mao, 2021). The basic principle of microwave heating is that microwave energy causes polar molecules such as water, fats, and sugars also the ionic compound in the material to rapidly rotate and generate heat due to the frictional forces generated by this motion. This rotational motion generates heat throughout the material, which could be used to cook or heat the material (Xue et al., 2018). There are some disadvantages of microwave heating technique reported by Joseph et al. (2017) where this method could sometimes result in uneven heating, with some areas of the material getting hotter than others. This could lead to overcooked or undercooked food or uneven heating of materials. However, microwave heating is a rapid and efficient heating method if compared to the conventional method.

There is various method could be used to extract sacha inchi oil from the seed and the most common are cold pressing method and solvent extraction. Compared to cold pressing method, solvent extraction provides higher oil yields but extracting solvents are toxic and flammable. The consumption of toxic solvent is facing restriction in the food industry and need to be reduced into a very small concentrations, causing higher cost (Jitpinit et al., 2022). To overcome this limitation, researchers nowadays are searching for the alternative method.

The aqueous enzymatic extraction (AEE) is a promising method for the simultaneous extraction of oil and protein from oilseeds with the assistance of enzymes. The AEE process has the advantages of having no organic solvent use, low energy consumption, and has mild reaction conditions (Liu et al., 2020). This method also could prevent the oxidation of polyunsaturated fatty acid (PUFA) in oil since it only needed a mild operating temperature (Nguyen et al., 2020). Despite all the advantages, the AEE method is considered as high cost which may attributed to the enzyme themselves because a significant amount is required. The non-availability of enzymes on a commercial scale has limited the development of such process, therefore choosing the right enzyme is important so that the drying process after the treatment can be eliminated. An added problem with AEE is that it is impossible to avoid emulsification of the extracted oil, which requires post-extraction de-emulsification to recover and enhance oil yield (Mat Yusoff et al., 2015). Among various enzymes used, the most suitable enzyme was papain. Papain destroys the protein, a protective layer of fat globules, so the extraction process results in higher oil yield (Rukman et al., 2018).

In the current study, papain enzyme is chosen to be used in AEE method for the extraction of the oil from sacha inchi seed. Pre-treatment on sacha inchi seed prior to oil extraction was done using microwave drying method. The effect of drying power mode and drying time during drying method on oil yield also were investigated. It is expected that the amount of phenolic compound in oil yield increases as drying power increased. AEE is considered as green and safe extraction method since none of hazardous chemicals or solvents are involved.

MATERIALS AND METHOD

Materials

Plukenetia volubilis L. (sacha inchi) seed was purchased from online supplier (Shopee). Papain enzyme was obtained from Nanning Doing Higher Bio-Tech Co. Ltd. The enzyme used was 1% (w/w, based on the seed weight). While for gallic acid, Folin-Ciocalteu reagent, ethanol, and anhydrous sodium carbonate were purchased from Sigma Aldrich.

Microwave Oven Drying Method

Microwave oven with 800 W was used for drying sacha inchi seed. Four different power modes namely, low, medium, medium-high, and high. The drying time for each drying power mode were varied at 5, 7, and 9 minutes. **Table 1** summarizes the sample designation drying conditions for the sacha inchi seed.

Table 1: Condition used to dry the sacha inchi seed.

Sample design	Power mode	Drying time (min)
Set 1	Low	5
Set 2	Low	7
Set 3	Low	9
Set 4	Medium	5
Set 5	Medium	7
Set 6	Medium	9
Set 7	Medium-high	5
Set 8	Medium-high	7
Set 9	Medium-high	9
Set 10	High	5
Set 11	High	7
Set 12	High	9

The weight of the kernels before and after drying process were recorded and the moisture loss was calculated using Eq. (1) below:

$$Moisture\ loss\ (\%) = \frac{w-d}{w} \times 100 \qquad \text{Eq. (1)}$$

where *w* is wet weight (g), and *d* is weight after drying (g).

The dried seed kernels were ground into smaller size and stored in a dark beg and refrigerated at 4 °C for 3 days to avoid oxidation and microbial growth (Nguyen et al., 2020). Otherwise, the enzyme used in the extraction later was sensitive to high temperatures.

Aqueous Enzymatic Extraction

The extraction procedure was followed Nguyen et al (2020) in their previous study. Firstly, 0.3 g of papain enzyme was dissolved in 60 mL of water. Then, 29.7 g of ground seed was added into the solution and the mixture was shaken at 200 rpm for 120 minutes at 50 °C using an incubator shaker. The conical flasks were covered using aluminium foil to avoid spilling. After that, the mixture was centrifuged for 30 minutes, 4000 x g at room temperature to separate the oil and water. The collected oil was added with anhydrous sodium sulphate before being heated at 105 °C in the oven for 3 hours. The oil yield was calculated using Eq. (2) below:

$$Oil\ yield\ (\%) = \frac{m}{d} \times 100 \qquad \text{Eq. (2)}$$

Where m is weight of extracted oil (g), and d is weight of sacha inchi seed (g).

Total Phenolic Compound (TPC) Analysis

Gallic acid stock solution was prepared by dissolving 0.5 g of gallic acid in 10 mL ethanol, then, distilled water was added up to 100 mL. After that, five working solutions of gallic acid were prepared at 0, 50, 100, 250, and 500 mg/L. Folin-Ciocalteu reagent was in yellow colour (good) conditions. 7.5 g anhydrous sodium carbonate was then added into 100 mL distilled water to form 7.5% sodium carbonate solution (Jin et al., 2019). To plot the standard curve, firstly, 3.16 mL distilled water was added into the test tubes with 0.2 mL Folin-Ciocalteu reagent, followed by 0.6 mL sodium carbonate solution and 40 µL of gallic acid stock solution. The mixture was shaken vigorously and incubated in the dark for 2 hours at room temperature. The absorbances of the standards is measured at $\lambda = 765$ nm using UV-Vis spectrophotometer. The samples were prepared in triplicate and the calibration curve was plotted. For TPC measurement, the oil was being replaced with 40 µL of gallic acid equivalents per 100 g of oil (mg GAE/100g oil).

Fatty Acid Analysis

The highest sacha inchi oil yield sample was chosen and test for the fatty acid content using reverse phase C_{18} high-performance liquid chromatography (HPLC) separation using a methanol/water eluent mixture and the value of omega-3 and omega-6 were determined using in house method based on AOAC 986.25 (E) 17th edition, 2000.

RESULTS AND DISCUSSION

In this section, results of moisture loss, oil yield, oil colour, and TPC content are presented and discussed. The highest oil yield has been selected to undergo fatty acid analysis which include Omega-3 and Omega-6.

Moisture Loss After Drying Process

The effect of drying power and drying time on moisture loss are shown in **Figure 1**. Each set of seed sample was dried according to the condition listed in **Table 1**. It is showing that the higher the drying power and longer the drying time, the higher the moisture loss of the sacha inchi seed. The result obtained agreed with the previous report by Zambrano et al. (2019). Set 7 and set 8 were having the almost similar moisture loss as the previous set 1, 2, and 3. It might be because of the nonuniform drying. The large particle size, the inner side needs more time to be heated (Li et al., 2011). The small particle size of the seed can be heated uniformly since the surface area is larger, the inner side can be heated at the same time.

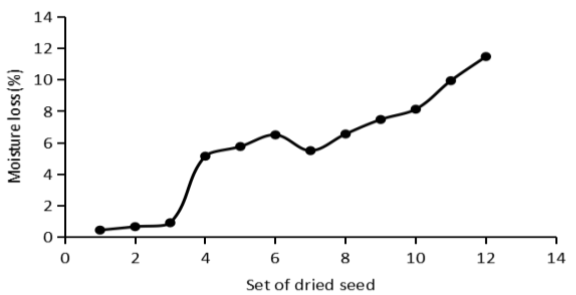

Figure 1 Total of moisture loss (%) for each set of dried seed prepared at different drying power and drying time.

Sacha Inchi Oil Yield

Figure 2 shows the amount of oil yield based on the set of dried set sample. The variable used for each set as listed in **Table 1**. Based on the figure, no production of sacha inchi oil were observed for seed samples that heated at lower drying power mode (set 1, 2, and 3). This might happen since these three sets of seed sample have the lowest moisture loss. Seeds with high moisture content could develops the mucilage on the outer walls which will swell later and resulting a cushioning effect thus prevent the oil expression (Aramu et al., 2016). Keneni et al. (2019) state in their report that no oil yield produces because of the oil hydrolysis, forming the free fatty acids. However, the most relevant reason for the result obtained in the current study is due to high moisture content that led to the formation of cloudy oil since it is emulsified with water (as shown in **Figure 2**).

The highest oil yield was obtained for the seed that dried at medium power (Set 4, 5, and 6). It because heating process have denatured and coagulated the seed protein. This results the viscosity and surface tension of the oil decreases, thus improves the fluidity of the oil in the seed resulting the oil is easier to be expelled (Zhang et al., 2021). As shown in **Figure 2**, the oil layer is appeared at the top after centrifuging since less emulsion happened.

While for other samples (medium-high drying power: set 7, 8 and 9) and (high drying power: set 10, 11, and 12) were produced lower oil yield. These results high possibly caused by the acceleration of oil volatilization. Some nutrients in the seed lost also experienced seed lipid oxidation (Wang et al., 2021). The lower oil yield and the loss of nutrients are being the reason of not choosing these extracted oils as qualified oil. Generally, it can be concluded that the drying power on seed influenced the amount of oil yield, and the optimum condition was agreed at medium drying power (to be specific is set 5 of dried seed sample).

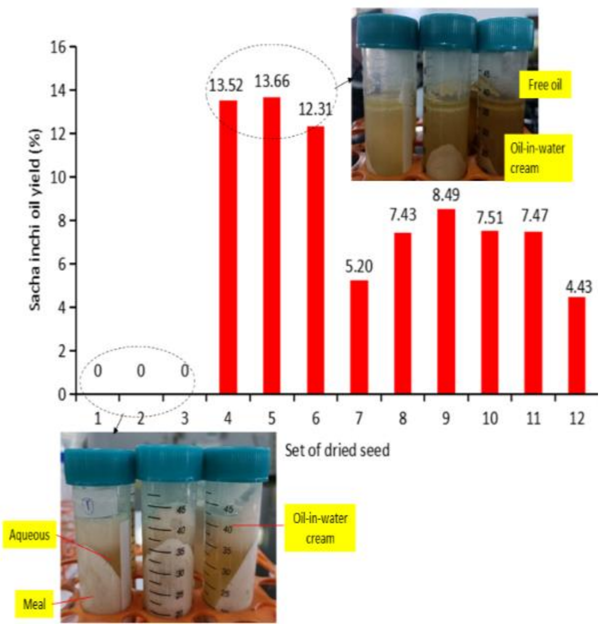

Figure 2 Sacha inchi oil yield (%) for each set of dried seed prepared at different drying power and drying time.

Sacha Inchi Oil Colour

Figure 3 shows the colour of the extracted oil is being darker when increasing the drying power and drying time. According to Suri et al. (2019), increasing the roasting temperature especially over—roasting, the level of 5-hydroxymethlfurfural (HMF) will also be increased. HMF is a chemical compound that is responsible for the browning of the oil. In the research of Zhang et al. (2021), the yellowish or red colour of the oil is due to the carotenoid pigments. When increasing the drying power and drying time, the carotenoid pigments will rise.

Figure 3 The extracted oil colour from lowest (left) drying power and drying time to the highest (right).

The Phenolic Content (TPC) of Sacha Inci Oil

Figure 4 shows that the total phenolic content of the oil rises with the increasing of drying duration. At fix drying power, the 9 minutes of drying time (Set 6, 9, and 12) are resulting the highest total phenolic content. Among the three sets stated just now, Set 12 is getting the highest TPC content which at 4.04 mg GAE/100 mg oil. According to Goyal et al. (2022), formation of phenolic compounds may be because of the improvement of the antioxidant activity due to roasting.

Since the high temperature soften the cellular structure of the seed, the phenolic compound will be more extracted. At the same time, Alide et al. (2020) states that high heating power can activate the polyphenol oxidase enzyme, thus inhibiting polyphenolics degradation, meaning that the phenolic compounds are maintained during the high heating process. Moreover, the reaction between reducing sugar and amino acid by addition of the heat possible to affect the TPC result, it is because the Maillard reaction product such as 5-hydrxymethylfurfural (HMF) is produced during the heating process (Zhang et al., 2021). HMF is one of the compounds under the phenolics group, however, this compound risks the human body's health (Tamanna and Mahmood, 2015). Therefore, Set 12 or brownish oil would not be a good consideration for edible oil.

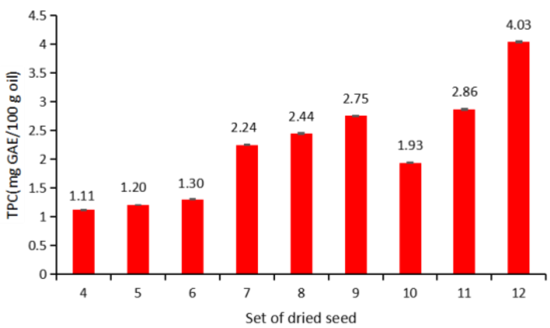

Figure 4 Total phenolic compound found in 100 g of oil extracted from each set of dried seed.

Fatty Acid of the Sacha Inchi Oil

Set 5 with medium drying power and 7 minutes drying time is selected to undergo the fatty acid analysis. The amount of omega-3 found in the oil was 0.002 g/100 g oil and no omega-6 was detected. The result obtained was quite low compared to previous study that using papain enzyme assisted extraction method (Nguyen et al., 2020). This result might happen due to fruit maturity stages. The mature sacha inchi poroduce high oil content and more fatty acids compared with immature (Yanti et al., 2022). Another factor affecting fatty acid content is heating temperature, which can change the geometrical double bond formation into trans- fatty acids. As we know, oils rich in omega 3 are sensitive to heat, oxygen, and light. Therefore, heating can trigger the oxidation of unsaturated fatty acids and caused the formation of hydrogen peroxide thus affecting the content of omega 3 and omega 6 in oil (Giuffré et al., 2020).

CONCLUSION

Based on the result obtained, set 5 of dried seed is selected as the best condition as it produce high yield of sacha inchi oil. The conditions for Set 5 is using the medium drying power and 7 minutes of drying time. At higher drying power and longer drying time, lower oil yield was recorded. For phenolic compound analysis, set 5 of dried seed sample shows a lower amount compared to set 12, however it is believed that the quality of the extracted oil is not affected. It is because the higher the drying power and the drying time, the easier to undergo lipid oxidation or oil volatilization. Additionally, the brownish colour and the high total phenolic compound of Set 12 may be because of the 5-hydroxymethylfurfural (HMF) which produced from Maillard reaction. Maillard reaction will affect the body's health by reducing the nutrition of the oil and producing acrylamide in oil which would leading to cancer (Tamanna and Mahmood 2015).

For further study, to improve the sacha inchi oil yield, the de-emulsification process is recommended to be added during the aqueous enzymatic extraction process. Adding the enzyme into the solution to hydrolyse the interfacial protein, then the protein molecular size and oil droplet interfacial rigidity can be decreased (Yusoff et al., 2016). Moreover, the factor that affects the oil extraction include the concentration of enzyme. The concentration of enzyme should be investigated to get the optimum oil yield (Kumar et al., 2017). To get a better moisture loss result, the seed size needs to be grouped into smaller particles sizes for uniform heating (Li et al., 2011).

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