Application of Enzymes for Coconut Oil Extraction

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Abstract. Coconut oil is edible oil extracted from endosperm of coconut (Cocos Nucifera) using different methods. In this report the coconut oil extraction from coconut meat rasp using pineapple enzymes were studied and compared with commercial enzymatic oil extraction. Pineapple enzymes were extracted from fresh pineapple fruit. The effectiveness of pineapple enzyme extract on coconut oil extraction from coconut meat rasp was studied at different enzyme concentrations of 0.5 to 2% (W/W) and pH of 4.5 to 7.5 with incubation times of 2 to 8 hr at 40 to 70 °C respectively. The commercial technical enzymes used in this study were from different companies (Valley enzyme®, Novozyme® and AB-enzyme®). Valley enzyme® at 1% (w/w) enzyme concentration and 1:1 enzyme solution to coconut rasp ratio found to be the most effective commercial enzyme in comparison with Novozyme® and AB-enzyme®. The optimum conditions for coconut oil extraction from coconut meat rasp using pineapple enzyme were at 1% (W/W) enzyme concentration, pH 6.5, 60 °C and 6 hr, while the optimum conditions for Valley enzyme were at pH 5.5, 60 °C and 6 hr. Oil yield obtained from Valley enzyme® and pineapple enzyme extract under optimum conditions were 21.89 % and 20.50%, respectively. Therefore, the pineapple enzyme extract is a promising natural alternative to commercial technical enzymes for coconut oil extraction.

1. Introduction

Aqueous enzymatic oil extraction is undoubtedly an emerging technology in the fats and oil industry since it offers many advantages compared to conventional extraction. For instance, it eliminates solvent consumption which reportedly may also lower investment costs [1], [2] and energy requirements [1]. The problem of low extraction efficiency of aqueous processes may be overcome by the use of hydrolytic enzymes which help release oil and increase the yield as some studies have shown. Besides this, environmental issues, especially the increasing concern about volatile organic compounds caused by solvent emissions, have also sharpened the focus on these pectinases to break the structure of cotyledon cell walls. Enzyme action makes the structure more permeable; the extent depends on particle size. Proteolytic enzymes mainly hydrolyze the proteins in the cell membranes as well as inside the cytoplasm [3]. Proteolytic enzymes can also affect the cytoplasmic network which is largely composed of proteins in the case of soybean and some other oilseeds, thereby making the inner structure less tightly bound and compact and thus enabling easier removal of protein and lipid from the cell [3]. The difference in the oilseed composition determines the choice of enzymes to be used for each oilseed or fruit. While rapeseed has a significant content of pectin, relatively lower levels are found in coconut, and corn germ. For instance, an enzymatic mixture which is tailored to hydrolyse the different substances should be used in order to obtain highest possible oil yields [4]. In the case of coconut, the extraction yield of coconut oil substantially increased by combined treatment of
polygalacturonase, α-amylase, and protease in an aqueous system, thereby obtaining final yields as high as 80%. The enzyme did not influence the emulsion stability during coconut oil extraction and the emulsion formed was very unstable resulting in rapid oil separation [5]. Soo et al. [6] have investigated the enzymatic extraction of virgin coconut oil from coconut endosperm using crude enzyme of pineapple fruit and compared with coconut oil extraction using mechanical, and ultrasound method. They found that using crude enzyme from pineapple fruit the oil yield was highest (77.7%) compare to mechanical (58.6%) or ultrasonic treated (24.1%). To date, there is no report on comparison the effectiveness of crude enzyme from pineapple fruit for extraction of coconut oil compare to on the market available technical enzymes. The aim of this study was to investigate the possibility of using pineapple enzyme extract as an alternative to commercial technical enzymes for increasing the oil yield from coconut.

2. Materials and methods

2.1. Raw materials
Coconut rasp from Market (Nakhornpathom/Thailand) was used for all experiments.

2.2. Enzyme extraction from pineapple
290 g fresh pineapple was cut in small pieces. Pineapple pieces were blended with 250 ml cold solution of 2 M Tris buffer (pH 9) containing 0.1 M NaCl in a household mixer. After filtration of blended pineapple through 2 layers of cheese cloth, the filtrate was mixed with ammonium sulphate (Merck, Germany) up to 25% saturation. This mixture was then centrifuged at 11,000 × g, 4 °C for 20 min. The supernatant was then mixed with ammonium sulphate up to 80% saturation and centrifuged again as above. The precipitated crude enzymes were collected and dialysed against cold distilled water using dialyse membrane (cut off 10000-20000, Roth, Karlsruhe, Germany). The dialysed enzyme was stored at +4 °C. For studying the effect of commercial enzymes on oil yield, mixtures of enzymes were prepared as follows (Table 1).

Table 1. Applied technical enzyme mixture from different companies for enzymatic treatment of coconut for enhanced oil extraction

| AB Enzyme Co, Ltd, Germany: | Co, Ltd, USA: | Novozyme Co, Ltd, Denmark: |
|-----------------------------|---------------|--------------------------|
| Rohapect max 25%            | Validase GA 25% | Alcalase 2.5 L 25%       |
| Rohapect D52 special 25%    | Validase HT 340L | Viscozyme L 25%         |
| Rohapect DA 122 25%         | Validase ANC-L 25% | Celluclast 1.5 L 25%    |
| Rohapect CL 25%             | Crystalzyme 25% | Spirizyme Fuel 25%      |
|                             | 200 XL 25%    | Liquizyme SD DS         |

2.3. Experiments

2.3.1. Coconut extraction by traditional method. 25 g of dried coconut rasp was mixed with 50 g water and then heated at 90 °C for 4 h in a water bath. The heated sample was cooled and stored for 2 days at room temperature. Finally, the sample was pressed and the oil content was recorded.

2.3.2. Coconut extraction by soxhlet method. 20 g of fresh coconut rasp was dried in oven at 105 °C for 120 min and then insert in soxhlet equipment. Oil extraction was carried out using hexane solvent for 6 h. The total fat content was calculated gravimetric after evaporating the hexane from the extract.

2.3.3. Coconut oil extraction using enzymes. A) To study the optimum condition for commercial enzymes activity: 50 g of coconut rasp was mixed with 50 ml of distilled water and placed in a beaker.
No enzyme or 1% w/w (1 g in 100 g mixture of coconut and water) of AB Enzyme mixture or 1% w/w Novozyme enzyme mixture or 1% w/w Valley enzyme was put into each beaker. All the beakers were incubated in a water bath at 50 °C for 4 h or at temperature of 40 to 70 °C (for investigation of temperature effect) and 2 to 8 h (for investigation of treatment time). Afterwards the samples were cooled to room temperature (30 °C) and pressed in a laboratory hydraulic press. The coconut milk was then centrifuged at 4000 × g, 5 °C for 30 min. The cream phase after centrifugation was separated and heated at 50 °C for 4 h. Finally the heated cream was centrifuged again at 4000×g, 30 °C for 20 min to obtain the coconut oil. The separated oil was calculated gravimetric. For studying the effect of pH effect on oil yield using commercial enzymes the same steps as above were carried out but by varying the pH (pH of coconut/ enzyme mixture from 4.5 to 7.5) (figure 1). B) to study the optimum condition for pineapple enzyme extract activity: The preparations of samples were similar to 2.3.2 A, except instead of commercial enzymes used in to 2.3.2 A was pineapple enzyme extracts at concentrations of 0 (no pineapple enzyme added); 0.5; 1.5; 2.0; 2.5 % w/w used.

2.3.4. Equipment. The untreated or enzyme treated samples were pressed in a laboratory scale hydraulic press. The pressure during pressing was 40 bar and the press duration was 5 min constant at room temperature

3.Results and discussions

3.1. Comparison the activity of different commercial enzyme mixture
Commercial enzyme mixtures from different companies used in this study have shown nearly the same oil yield increase. Using 1% w/w commercial enzymes increased the oil yield from approx. 12% (without enzyme) to approx. 18% after 4h incubation at 50 °C. Comparing the commercial enzymes showed that the Valley enzymes mixture is more effective than AB enzyme and Novozymes at given treatment conditions (figure 1). Because of higher activity of Valley enzyme mixture compared to other commercial enzyme mixtures used in this study (AB enzyme and Novozyme), the following experiments were carried out with Valley enzymes and pineapple enzyme extract.

3.2. Effect of pineapple enzyme extract on coconut oil yield
Adding pineapple enzyme in a concentration of 1% w/w (incubation time 4 h at 50 °C) leads to distinctly higher oil yield compared to samples without adding enzyme. The oil yield after treatment with pineapple enzyme was nearly the same as in the case of treatment with the Valley enzyme and 1% w/w enzyme concentration (figure 2).
3.3. Effect of pineapple enzyme concentration on oil yield
Increasing the pineapple enzyme concentration from 0.5% w/w to 1.0% w/w increased the oil yield rapidly. Whereas the oil yield without adding enzyme was very low (approx. 12.5 %), the oil yield increased up to 17.8% after adding 1% pineapple enzyme extract at treatment time of 4 h at 50 °C and pH 4.5. Further, increasing the pineapple enzyme concentration showed no oil yield increase more (figure 3). Comparison the oil yield using pineapple enzyme extract and Valley enzyme at concentration of 1%, 50 °C and incubation time of 4h have shown that the oil yield using pineapple enzyme extract was lower compare to yield on Valley enzyme treated sample (figure 4).

3.4. Effect of pH on oil yield
The oil yield was higher at pH 6.5 for sample treated with pineapple enzyme compare to pH 4.5. At pH 6.5 the highest oil yield of 18.5 % was observed. Further increasing the pH to 7.5 showed negative effect on oil yield (figure 4). In contrast, the Valley enzyme mixture showed the maximal activity at pH 5.5 and treatment temperature of 50 °C (figure 4).

3.5. Effect treatment temperature on oil yield
Increasing the treatment temperature during incubation from 40°C to 50 °C increased the enzyme activity of the Valley enzyme. The maximum oil yield of 20.97% could be achieved after 4 h treatment at 50 °C and pH 5.5. In contrast, the pineapple enzyme extract showed the highest activity at a temperature of 60 °C. Up to 20 % oil yield could be observed after treatment of coconut at 60 °C, 4h and 1% pineapple enzyme extract at pH 6.5 (figure 5).

3.6. Effect of treatment time on oil yield
The oil yield during enzyme treatment of coconut was dependent on treatment time as shown 6. Increasing the treatment time from 2 h to 4 h increased the oil yield for Valley enzyme as well as the pineapple enzyme treated samples. Further increasing the treatment time up to 8 h had only a negligible positive effect on oil yield in the case of pineapple enzyme extract at optimal treatment conditions (pH 6.5, 60 °C, for the pineapple enzyme and pH 5.5, 50 °C for the Valley enzyme) (figure 6).
3.7. Comparison the effect of different enzymes on oil yield

The comparison of oil yield between traditional methods, control (without adding enzyme) and solvent extraction (soxhlet method) or with pineapple enzyme extract extract and Valley enzyme showed that the pineapple enzyme extract was a suitable alternative to commercial enzymes as shown in figure 7. Up to 70% oil compare to organic solvent extraction (soxhlet) method could be extracted using 1% w/w pineapple enzyme extract at optimal treatment conditons (pH 6.5, 60 °C, 4h). The fruit pineapple, Ananas comosus (L) Merr. is a rich source of a mixture of cysteine proteinase, the most abundant among them being bromelaine (EC 3.4.22.33), which hydrolytically cleaves the internal peptide bonds in proteins with relatively broad specificity [7]. Singh et al.[8] have investigated the application of pineapple extract for oak tasar (Antheraea proylei J.) silk cocoon cooking. They have found that the optimum activity of pineapple proteinase is at 60 °C. The reason for increasing of oil yield of coconut meat rasp by using pineapple enzyme is maybe due to proteolytic and pectolytic enzyme in pineapple enzyme extract. It is postulated that pineapple extract contains proteoletic enzyme (Bromelain), that could facilitate the the relase of coconut oil from coconut milk emulsion [6]. Further investigations are ongoing to determine the pectolytic and proteolitic enzyme activity in pineapple enzyme extract.

4. Conclusion

The oil yield of fresh coconut rasp by using pineapple enzyme extract was nearly similar to commercial enzyme (Valley enzyme) and distinctly higher than samples without enzyme treatment (control). The optimal conditions for coconut oil extraction using pineapple enzyme extract was (at 1:1
coconut meat: enzyme solution) at pH 6.5, 60 °C and 6h treatment time. The oil yield after enzyme treatment of coconut raspsi using pineapple enzyme extract was 20.5% (w/w) on wet-weight basis at optimal conditions. The optimal condition for Valley enzyme (at 1:1 coconut meat: enzyme solution) and 1% enzyme concentration was at pH 5.5, 50 °C, and 6h. The oil yield was 21.19% (w/w) in the case of Valley enzyme on wet-weight basis at optimal conditions. These data have showed that the pineapple extract as a natural enzyme source was suitable alternative to commercial technical enzymes for increasing the coconut oil yield.

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