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Purification of bromelain-extracted virgin coconut oil through activated carbon and cuttlefish bone: A comparison

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Abstract

Enzymatic treatment in producing virgin coconut oil (VCO) may result in impure oil quality due to the manufacturing and storing processes that often cause damage, and decrease quality. VCO produced using pineapple extract as source of enzyme was green, caused by the green colour of the pineapple's leaves, peels, and crowns. Therefore, the present work aimed to improve the quality of bromelain-extracted VCO by purifying and examining its appearance, moisture content, and free fatty acid content. Activated carbon and powdered cuttlefish bone were used as the purifying agent/adsorbent. VCO purification was done in a glass beaker by adding 0.05 g of adsorbent into the 50 mL of VCO, and stirred for 30 min at various temperatures (30, 40, and 50°C) to obtain the best setting for purification. The appearance, moisture content, and free fatty acid content were tested to analyse the quality of VCO before and after purification. The colour, moisture content, and free fatty acid content diminished during purification with both adsorbents. The most substantial reduction was for moisture content. The activated carbon was superior in diminishing colour and free fatty acid content, but the cuttlefish bone exceled in lowering moisture content. Double steps purification may be needed to improve the overall quality of bromelain-extracted VCO.

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Introduction

Virgin coconut oil (VCO) is a processed product from coconut fruit with health benefits, such as reducing cardiovascular diseases, anti-diabetic, and wound healing (Babu *et al*., 2014). VCO is produced by many methods, such as mechanical methods, fermentation, and enzymatic reactions (Satheesh, 2015). The enzymatic reaction was reported as the most environmentally friendly production method since it does not include heating and chemical application (Harimurti *et al*., 2020). There are many natural enzymes used in the production of VCO, such as bromelain dan papain (Iskandar and Edison, 2015; Perdani *et al*., 2019; Adi and Prayitno, 2019; Harimurti *et al*., 2020; 2022; Rohyami *et al*., 2022; Roni *et al*., 2022). The enzyme hydrolyses the protein emulsifier, and then the oil separates from the emulsion (Mat Yusoff *et al*., 2015; Li *et al*., 2017; Zang *et al*., 2019).

The steps in manufacturing VCO sometimes cause damage or a decrease in quality (Harni and Putri, 2014; Parlindungan *et al*., 2020). Taste and smell that turn rancid are caused by the hydrolysis reaction due to the high moisture content in VCO. The hydrolysis of fats can increase the acidity of the oil, while the oxidation of fats can increase the rancidity of the oil (Srivastava and Semwal, 2015; Ghosh *et al*., 2016; Dewi Natalia *et al*., 2019). One way to reduce moisture and oil peroxide levels is to use adsorbents in the VCO filtering. Oil quality is tested for moisture and free fatty acid contents (Parlindungan *et al*., 2020). Excessive moisture and free fatty acid contents in VCO can accelerate rancidity in the VCO product. Therefore, there is a need to identify another method that can hold the moisture and free fatty acid contents of VCO (Whitaker, 2018).

A previous paper (Subroto *et al*., 2021) reported the usage of activated carbon in the

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purification of bromelain-extracted VCO that produced a better quality of VCO than activated zeolite. Activated carbon has high absorption due to its pore volume, and can absorb gas and residue in solution (Laos *et al*., 2016; Parlindungan *et al*., 2020). Besides activated carbon, cuttlefish bone is also commonly used as an adsorbent, which is expected to be a promising adsorbent for VCO. Calcium carbonate and chitosan are considerable content of cuttlefish bone (Krishnan *et al*., 2021; Ait Hamdan *et al*., 2024; Elkhenany *et al*., 2024). Meanwhile, calcium carbonate and chitosan have been proven to be excellent adsorbents (Haji Azaman *et al*., 2018). The chitosan in cuttlefish bones has hydroxyl, amide, and amine groups (Baláž, 2021). Those compositions are characteristic of the formation of free chitosan, which makes this polymer polycationic. Therefore, this polymer is suitable for sewage treatment, medicine, food processing, and biotechnology (Riaz Rajoka *et al*., 2019; Martau *et al*., 2019; Pal *et al*., 2021; Krishnan *et al*., 2021; Udayakumar *et al*., 2021; Fatullayeva *et al*., 2022; Elkhenany *et al*., 2024). Due to its chemical composition containing hydroxyl groups and low utilisation (Siregar *et al*., 2017), cuttlefish bones were used as a moisture absorber in the present work. Moreover, Hasmath Farzana and Meenakshi (2014) revealed that cuttlefish bone powder is suitable as a dye adsorbent. Thus, powdered cuttlefish bone may improve the quality of bromelain-extracted VCO. The availability of cuttlefish and its low price were also considered for its utilisation in the present work.

The present work demonstrated the purification procedure of bromelain-extracted VCO utilising activated carbon and powdered cuttlefish bone to enhance the quality of VCO. The purification effectiveness was evaluated based on appearance, free fatty acid content reduction, and moisture content post-purification.

Materials and methods

Materials

The bromelain-extracted VCO was produced following a previous work on VCO extraction using bromelain enzyme from pineapple waste (Harimurti *et al*., 2022), and the commercial VCO obtained from Halmahera Maluku, Indonesia, was used as the standard. The activated carbon tablet was purchased from PT Eagle Indo Pharma. Raw cuttlefish bone was obtained from PASTI traditional market in Yogyakarta, Indonesia. Analytical grade oxalic acid and analytical grade sodium hydroxide were purchased from Merck Germany. Distilled water and analytical grade ethanol 96% were obtained from Bratachem, Indonesia.

Methods

Preparation of adsorbent

The carbon was powdered using a pestle and mortar, and then sifted using a 60-mesh sieve. The activation was done in the oven at 120°C for 4 h (Kemenkes, 2014). This activated carbon was then ready to be used for purification. The raw cuttlefish bone was cleaned using distilled water to remove impurities such as salt and remaining sand. Next, the cuttlefish bone was boiled for 10 min, followed by drying at 100°C for 24 h. Dried cuttlefish bone was powdered using a pestle and mortar, and then sieved using a 35-mesh sieve. Later, the cuttlefish bone powder was directly used as an adsorbent without further treatment.

Purification and determination of bromelain-extracted VCO quality

The VCO purification method was performed according to Haji Azaman *et al*. (2018). About 0.05 g of activated carbon or cuttlefish bone powder was mixed into 50 mL VCO, and stirred for 30 min at various temperatures (30, 40, and 50°C) in each experiment. Further, the mixture was left to settle for 24 h, followed by filtering using a filter paper. Appearance, moisture content, acid number, and colour reduction were tested to determine the quality of the oil. Indonesian National Standard was used to analyse the appearance of the treated VCO (SNI, 2008). The moisture content analysis was performed according to Nodjeng *et al*. (2013). The percentage of colour reduction was analysed using a spectrophotometer. The measurement was conducted by scanning the absorbance before and after purification at 200 - 800 nm for each bromelain-extracted VCO type. The results of the maximum wavelength test of each sample are shown in Figure 1 which shows that the maximum absorption was at 412.5 nm for bromelain-extracted VCO (crowns, leaves, and peels). The maximum wavelength of the bromelain-extracted VCO (stems) was not detected since the VCO was colourless. The fatty acid content

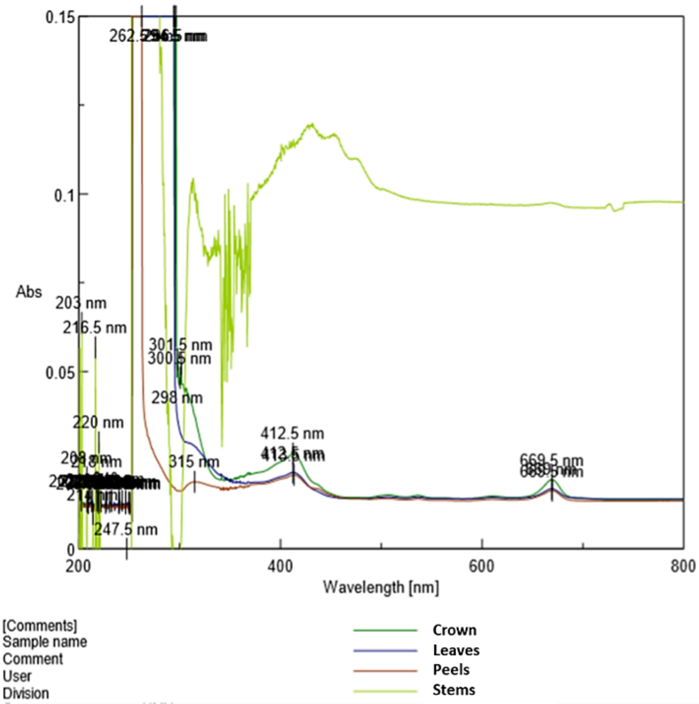


Figure 1. Maximum wavelength spectrum profile of VCO made with the help of enzymes from the extracts of pineapple's crowns, leaves, peels, and stems.

was tested using volumetric analysis with phenolphthalein as the indicator (Nodjeng *et al*., 2013). A statistical paired sample *t*-test was conducted to determine the effectiveness of purification, and whether there were any significant differences between the quality of VCO before and after purification using activated carbon or cuttlefish bone.

Results and discussion

Organoleptic test

The organoleptic test was carried out by looking at the colour, smell, and texture of the VCO (Negara *et al*., 2016). Based on the observation, the smell of the bromelain-extracted VCO was like coconut, and the texture was slightly thin. The results of colour change tests before and after purification with activated carbon and cuttlefish bone, respectively, are presented in Table 1.

Table 1 shows the colour reduction when the bromelain-extracted VCO (pineapple's crown and leaves) was purified using activated carbon. The same observation was made when the bromelain-extracted VCO (leaves) was purified using powdered cuttlefish bone. The fresh pineapple enzyme extracted from peals contains carotenoid pigments, namely carotene and xanthophylls, and coloured yellow, orange, and red (Steingass *et al*., 2020). This colour reduction may be due to activated carbon binding the colour and cuttlefish bone (Hadavifar *et al*., 2016).

Table 2 shows the absorbance results for each sample at various purification temperatures using activated carbon and cuttlefish bone. In the bromelain-extracted VCO (crown and leaves), the decrease in absorbance percentage before and after purification was different, and the colour was more transparent after purification at 50°C compared to before purification. However, the colour changes were not observed for bromelain-extracted VCO (peels and stems). Based on the results, increasing temperature reduced the absorbance reading. The data indicated that more purities present during the process were more absorbed in the activated carbon at higher temperatures. Higher temperatures would cause the empty sites for adsorption on activated carbon to be more numerous (Aljeboree *et al*., 2017). For cuttlefish bone, the higher adsorption at higher temperatures was due to the fast diffusion of purities through the external boundary layer, and the internal pores of the adsorbent. The fast diffusion was due to less resistance offered by viscous forces in the VCO (Parvin *et al*., 2021).

Table 1. Colour changes of bromelain-extracted VCO (crowns, leaves, peels, and stems of pineapple) before and after purification using activated carbon and cuttlefish bone.

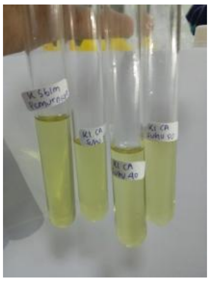
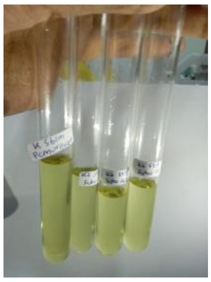
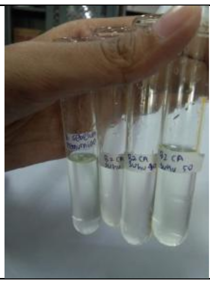
Enzyme source	Purification period	Result			
		Activated carbon		Cuttle fish bone	
		Colour		Colour	
Crown	Before purification	Greenish clear		Greenish clear	
	30°C	Clear green		Clear green	
	40°C	Clear yellow		Clear green	
	50°C	Clear		Greenish clear	
Leaf	Before purification	Greenish yellow		Greenish yellow	
	30°C	Greenish yellow		Greenish yellow	
	40°C	Greenish yellow		Greenish yellow	
	50°C	Greenish clear		Greenish clear	
Peel	Before purification	Slightly greenish yellow		Slightly greenish yellow	
	30°C	Slightly greenish yellow		Slightly greenish yellow	
	40°C	Slightly greenish yellow		Slightly greenish yellow	
	50°C	Slightly greenish yellow		Slightly greenish yellow	
Stem	Before purification	Clear		Clear	
	30°C	Clear		Clear	
	40°C	Clear		Clear	
	50°C	Clear		Clear	

Table 2. Absorbance of bromelain-extracted VCO (crown, leaf, peels, and stem of pineapple) before and after purification using activated carbon and cuttle fish bone.

Sample		Mean of maximum absorbance		% Decrease of maximum absorbance	
		Activated carbon	Cuttle fish bone	Activated carbon	Cuttle fish bone
Crown	Before purification	1.0231	1.0231	-	-
	30°C	0.7041	0.8261	31.1798	19.2552
	40°C	0.6305	0.6214	38.3980	39.2630
	50°C	0.2182	0.4645	78.6755	54.6037
Leaves	Before purification	0.9127	0.9127	-	-
	30°C	0.7716	0.8689	15.4651	4.8044
	40°C	0.7344	0.7515	19.5344	17.6674
	50°C	0.2444	0.5041	73.2231	44.7683
Peels	Before purification	0.6617	0.6617	-	-
	30°C	0.6456	0.6327	2.4331	4.3827
	40°C	0.6362	0.6077	3.8537	8.1684
	50°C	0.5883	0.5825	11.9692	11.9692
Stems	Before purification	0.1444	0.1444	-	-
	30°C	0.1098	0.1169	23.9612	19.0789
	40°C	0.1049	0.1049	27.3546	27.3199
	50°C	0.0672	0.0914	53.4626	36.7382

Moisture content

Moisture content is the amount of water in the material expressed as a percentage that evaporates on heating using an oven at a specific temperature and time (Negash *et al*., 2019). Based on Table 3, the average moisture content decreasing after purification using activated carbon and cuttlefish bone was more than 90% at 50°C. The ability of activated carbon to adsorb water is due to the availability of pores. Meanwhile, for cuttlefish, it is due to the availability of pores and chitin which contains hygroscopic functional groups of hydroxyl and amine (Cahyono, 2018). Therefore, the cuttlefish bone showed a higher ability to adsorb water in the VCO. The polar groups adsorb water by hydrogen bonding (Walke *et al*., 2014). The water adsorption was also higher at higher temperatures, as the viscosity of VCO decreased, so oil diffusion into the pores became faster (Parvin *et al*., 2021). Moreover, the formation of hydrogen bonds may be faster at higher temperatures since the activation energy increases at higher temperatures (Vyazovkin, 2016).

Free fatty acid

Free fatty acid content is the percentage of free fatty acids in the oil, which is determined by base neutralisation (Vicentini-Polette *et al*., 2021). High content of free fatty acids in the oil indicates a decrease in its quality. Therefore, these free fatty acids can indicate oil damage that might arise from the hydrolysis reaction due to water in the oil. Table 4 shows the test results for free fatty acids before and after purification using activated carbon and cuttlefish bone. Free fatty acid concentration decreased by adsorption using activated carbon or cuttlefish bone. The adsorption ability of activated carbon was higher than that of the cuttlefish bone. The lower activities in cuttlefish bone may be due to the hydrophilicity of chitin, leading to less adsorption ability (Omer *et al*., 2021). The higher adsorption of free fatty acid by activated carbon may be because the free fatty acids came into the pores available on the surface of the carbon (Guliyev *et al*., 2018). Higher adsorption was also found at higher temperatures; this may be due to the decrease in the viscosity of VCO at higher temperatures, thus causing faster diffusion into the pores (Parvin *et al*., 2021).

Statistical analysis

Paired sample *t*-test is one of the testing methods used to assess a treatment's effectiveness. It is characterised by the difference in the average before and after the treatment is given (Kim, 2015). Therefore, in the present work, a paired sample *t*-test

Table 3. Results of moisture content analysis in bromelain-extracted VCO (crown, leaves, peels, and stems of pineapple) before and after purification using activated carbon and cuttle fish bone.

Sample		Mean of water content (%)		%Decrease in water content	
		Activated carbon	Cuttle fish bone	Activated carbon	Cuttle fish bone
Crown	Before purification	4.3455	4.3455	-	-
	30°C	0.3075	0.5722	92.9235	86.8329
	40°C	0.2078	0.4223	95.2189	90.2825
	50°C	0.1822	0.3646	95.8063	91.6107
Leaves	Before purification	2.6671	2.6671	-	-
	30°C	0.3136	0.1938	88.2421	92.7339
	40°C	0.2463	0.1868	90.7635	92.0619
	50°C	0.1885	0.0830	92.9327	96.8870
Peels	Before purification	4.4642	4.4642	-	-
	30°C	2.6496	0.3606	40.6486	91.9226
	40°C	1.3609	0.3395	69.5155	92.3951
	50°C	0.4186	0.3312	90.6236	92.5810
Stems	Before purification	6.1953	6.1953	-	-
	30°C	3.7416	0.8485	39.6054	86.3045
	40°C	0.8871	0.4763	85.6814	92.3116
	50°C	0.8526	0.2578	86.2379	95.8386

Table 4. Results of free fatty acid levels analysis of bromelain-extracted VCO (crown, leaves, peels, and stems of pineapple) before purification and after purification using activated carbon and cuttle fish bone.

Sample		Mean of FFA level (%)		%Decrease of FFA level	
		Activated carbon	Cuttle fish bone	Activated carbon	Cuttle fish bone
Crown	Before purification	3.4071	3.4071	-	-
	30°C	0.2866	2.4282	91.5873	28.7302
	40°C	0.1947	2.3796	94.2857	30.1587
	50°C	0.1893	2.3525	94.4444	30.9524
Leaves	Before purification	4.9474	4.9474	-	-
	30°C	0.4975	3.4233	89.9433	30.0806
	40°C	0.2379	3.3368	95.1903	32.5547
	50°C	0.2217	3.2989	95.5182	33.3198
Peels	Before purification	6.1544	6.1544	-	-
	30°C	5.5325	5.5703	10.1055	9.4903
	40°C	5.2729	5.3865	14.3234	12.4780
	50°C	4.9755	5.3811	19.1564	12.5659
Stems	Before purification	1.9269	1.9269	-	-
	30°C	1.5738	1.8123	18.3264	5.9459
	40°C	1.4926	1.7289	22.5364	10.2703
	50°C	1.4169	1.7082	26.4657	11.3514

was conducted to determine the effectiveness of the treatment of providing adsorbents (activated carbon and cuttlefish bone) on the purification of bromelain-extracted VCO (crowns, leaves, peels, and stems of pineapple). The best temperature for the purification was 50°C. Therefore, the statistical analysis was carried out for the data at 50°C. The effectiveness was observed by the difference in the average test results of absorbance, moisture content, and free fatty acid content before and after purification. Tables 5 - 8 show the results of the paired sample *t*-test.

Table 5. Paired sample correlations before and after purification using activated carbon.

		N	Correlation	Sig.
Pair 1	Absorbance before and after activated carbon purification	4	.302	0.698
Pair 2	Moisture content before and after activated carbon purification	4	.876	0.124
Pair 3	Free fatty acid level before and after activated carbon purification	4	.574	0.426

Table 6. Paired samples *t*-test before and after purification using activated carbon.

		N	Correlation	Sig.
Pair 1	Absorbance before and after cuttlefish bone purification	4	0.812	0.188
Pair 2	Moisture content before and after cuttlefish bone purification	4	0.621	0.379
Pair 3	Free fatty acid level before and after cuttlefish bone purification	4	0.949	0.051

Table 7. Paired sample correlations before and after purification using cuttlefish bone.

		df	Sig. (2-tailed)
Pair 1	Absorbance before and after activated carbon purification	3	0.126
Pair 2	Moisture content before and after activated carbon purification	3	0.006
Pair 3	Free fatty acid level before and after activated carbon purification	3	0.088

Table 8. Paired sample *t*-test before and after purification using cuttlefish bone.

		df	Sig. (2-tailed)
Pair 1	Absorbance before and after activated carbon purification	3	0.114
Pair 2	Moisture content before and after activated carbon purification	3	0.008
Pair 3	Free fatty acid level before and after activated carbon purification	3	0.053

The normality test found a significance value of > 0.05, indicating that the data were normally distributed. The normality test results before and after purification with activated carbon were normally distributed data showed by sig > 0.05. A paired sample *t*-test was then performed as the data were normally distributed. Based on the statistical tests using the paired-sample *t*-test, which compared the results before purification with after purification of activated carbon and cuttlefish bone with the best temperature of 50°C in four samples (crowns, leaves, peels, and stems), the present work was concluded.

The result of the absorbance test (sig > 0.05, namely 0.126 and 0.114) and the free fatty acid content (sig > 0.05, namely 0.088 and 0.053) showed that the H0 was accepted, and H1 was rejected. The result indicated no significant difference between the average values before purification and the average value after purification. These results were not significant as the VCO produced with the help of enzymes that were extracted from the crowns, leaves, peels, and stems extract of pineapple did not show real colour change and high free fatty acid levels due to the fatty acid content in cuttlefish bones as previously discussed. Meanwhile, in the moisture content test (sig ≤ 0.05, namely 0.006 and 0.008), H1 was accepted, and H0 was rejected. This result indicated a significant difference between the average value before purification and the average value after purification. These results were obtained because in the four samples tested, the decrease in moisture content was significant from before to after purification. Based on the purification results, the purification should not be carried out using one purification method. A combination purification method may need to be conducted to achieve high VCO quality.

Conclusion

Based on the obtained results, it can be concluded that activated carbon and cuttlefish bone could be used to purify bromelain-extracted VCO (crowns, leaves, peels, and stems of pineapple). The best temperature for purifying bromelain-extracted VCO using activated carbon and cuttlefish bone was 50°C. This was shown by the colour change results, which became clear or transparent greenish on the crowns and leaves of the pineapple. The colour, moisture content, and free fatty acid content decreased after purification using both adsorbents. However, the most significant decrease was the moisture content. The activated carbon was more effective in reducing the colour and free fatty acid, while the cuttlefish bone was more effective in reducing the moisture content. The present work recommends further purification of the VCO using two continuous methods of purification: cuttlefish bone initially to adsorb the water, and activated carbon subsequently to remove the colour and free fatty acid.

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