

Physical and Chemical Characteristics of Green Algae *Halimeda opuntia* Calcium Powder Extracted Using Hydrochloric Acid

Rania S. Nirwasita, Ima Wijayanti^{*}, and Apri D. Anggo



¹ Department of Fish Products Technology, Universitas Diponegoro, Semarang 50275, Indonesia

^{*}Corresponding Author:
imasetianto@gmail.com

Received: 6 November 2023

Accepted: 13 May 2024

Published: 31 May 2024

Academic Editor:

Prof. Dr. Ing. Azis Sitanggang, S.TP, M.Sc.

©Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology, 2024.
Accreditation Number: 148/M/KPT/2020.
ISSN: 2089-5690, e-ISSN: 2406-9272.
<https://doi.org/10.15578/squalen.857>

Abstract

Halimeda opuntia is one of the macroalgae species whose utilization is still slightly known. In the open sea, *H. opuntia* holds an important role in changing carbon dioxide (CO₂) to calcium carbonate (CaCO₃). This led to previous research analyzing the calcium content of *H. opuntia*, which showed 30.30%. However, the information about calcium powder extraction using HCl from *H. opuntia* has not been reported. This research aimed to observe the physical and chemical characteristics of calcium obtained from *H. opuntia* extracted using different hydrochloric acid (HCl) concentrations. The method used in this research was experimental laboratories with a completely randomized design (CRD) using different HCl concentrations (1N, 2N, and 3N). The extraction results were analyzed for yield value, moisture, ash, fat, protein, carbohydrate, calcium, color, Scanning Electron Microscope (SEM), particle size, and X-Ray Diffraction (XRD). Data were then analyzed using Analysis of Variance (ANOVA). The use of different HCl concentrations showed significant effect ($p < 0.05$) on ash content ($91.95 \pm 0.47\%$), carbohydrate content ($7.26 \pm 0.57\%$), lightness (L^*) (86.21 ± 0.83), yellowness or blueness (b^*) (8.81 ± 0.02), ΔE (11.33 ± 0.54), and whiteness index (WI) ($83.62 \pm 0.70\%$), where the calcium extracted by 1N HCl (CH1) showed the best result. The SEM microstructural analysis showed a non-porous surface and a single cubical shape in CH1. Diffractogram pattern of calcium extracted from *H. opuntia* using different concentrations of HCl done by XRD exhibited the same pattern as CaCO₃ for all samples. HCl solvent at 1 N can produce calcium powder from *H. opuntia* with high calcium content and better color characteristics.

Keywords: calcium carbonate, extraction, *Halimeda opuntia*, hydrochloric acid

Introduction

Calcium is important for human health, especially bone and teeth development. Natural calcium can be obtained from dairy products, including milk, cheese, yogurt, seeds, nuts, etc. However, dairy products such as milk as calcium sources have a risk for some people who are lactose intolerant. Hence, other organic materials, fishbone, and non-economic macroalgae can be an alternative source of calcium. Fishbone has been the most frequently used material for calcium extraction for a long time because of its high calcium content. However, using fishbone-based calcium often faces problems, especially in the odor because of the sometimes left fat. Some kinds of compounds can cause a fishy odor in fishbone calcium, such as 2-methyl-butanal, 3-methyl-butane, pentanal, and hexanal (Wijayanti et al., 2022). Thus, other natural resources with less fishy odor due to their low-fat content, such as *Halimeda opuntia*, can be the alternative to fishbone

calcium. *H. opuntia* is one of the high-calcium-containing green algae that grows perfectly in Indonesian water because of its tropical climate, yet exploration and utilization are still limited. *H. opuntia* is non-economic macroalgae. Utilization of this alga as a low-cost calcium source can increase its value. However, for sustainability production, the culture of this algae might be needed. *H. opuntia* is important in the ocean, altering carbon dioxide (CO₂) to calcium carbonate (CaCO₃). Calcareous algae often mention *H. opuntia* because the lime adhered to the segments due to CO₂ alteration. The important role of *H. opuntia* is altering CO₂ into CaCO₃. This indicates that it contains a high content of calcium. A previous study by Kepel et al. (2021) showed that *H. opuntia* contained 30.30% of calcium. However, information about calcium extraction from *H. opuntia* is unavailable.

There are several calcium extraction methods using solvents such as hydrochloric acid, acetic acid, and

citric acid (Kim et al., 2017; Rosnah et al., 2012); sodium hydroxide (Idowu et al., 2020), however hydrochloric acid (HCl) as the solvent for extraction is generally easy to do. The goal of extraction is to purify a compound from unwanted impurities. Mulyani et al. (2021) mentioned that the use of HCl showed its ability to remove the organic compound on the raw material used and impacted in increasing content of calcium. Agustini et al. (2011) showed that using HCl in extracting calcium could reduce the yield of calcium, while Sunardi and Krismawati (2021) showed an increasing amount of the yield in line with increasing HCl concentration. From the previous studies regarding calcium extraction, it can be concluded that the concentration of HCl used affected both chemical and physical characteristics.

HCl is an acid with the least dangerous effect compared to other acids, which is caused by its non-reactive and non-toxic chloride ions. Furthermore, HCl can dissolve many kinds of metals and produce chloride and hydrogen, increasing the solubility of water-insoluble compounds (Kurkinen et al., 2021). In calcium extraction, HCl will help to ease the process by opening the material's pores (Rosnah et al. 2021). Kusumawati et al. (2022) and Zainol et al. (2019) mentioned that using HCl in calcium extraction will dissolve the calcium and the organic compounds, such as protein. The existing correlation between the extraction method and using HCl to extract will benefit the calcium food enrichment (Asih et al. 2019).

Rosnah et al. (2021) reported that 4% HCl could be used for calcium extraction in chicken egg cells. However, the information about calcium powder extraction using HCl from seaweed, especially *H. opuntia*, has not been reported. Thus, it is important to conduct research in extracting and analyzing its characteristics to compare between calcium powder obtained from *H. opuntia* in this study with the well-known hydroxyapatite calcium from fishbone, which has been studied by other researchers (Wijayanti et al. 2020; Idowu et al., 2020; Benjakul et al., 2017). The exploration of *H. opuntia* calcium will also increase scientific information in particular. This study aimed to elucidate the impact of concentrations of HCl on the characteristics of calcium powder from seaweed *H. opuntia*.

Material and Methods

Material

Fresh *Halimeda opuntia* was collected and dried for three days by local fishermen in Rembang Central Java, Indonesia, with a moisture content of 33.5%. Other materials used in calcium extraction were

technical-grade hydrochloric acid (HCl), sodium hydroxide (NaOH), and distilled water.

Methods

Calcium Extraction

H. opuntia calcium extraction was done by following the method used by Habte et al. (2019) and Sunardi et al. (2020) with several modifications on the hydrochloric acid (HCl) and sodium hydroxide (NaOH) concentration used, as well as the calcination that was not done in this experiment. Dried *H. opuntia* was cleaned first from dirt and then ground into crude powder using a chopper (Han River HRJRJ-S2BK, China). Different concentrations of HCl were prepared by making 1N, 2N, and 3N solutions, which were then added to *H. opuntia* crude powder with a ratio of 1:20 (w/v). Extraction was done for 2 hours and at a temperature of 90°C using Waterbath (WiseBath WB-6, Daihan, Korea). The next step was filtration to get the filtrate and precipitate using 3N NaOH with a ratio of 1:1 (v/v). A white precipitate was formed at this stage, and the pH was still 13. Thus, decreasing the pH to 7 by changing the water was consequently done to ensure that the NaOH leftovers were removed. The neutralized sample was then dried using an oven (Mettler UN110, Germany) for 48 hours at a temperature of 110°C. The dried residue was then grounded using a high-speed grinder (Fomac FCT-Z200, Indonesia) for a minute and sieved with a 100 mesh-sized sieve. Samples were then named CH1, CH2, and CH3 for calcium extracted by 1N, 2N, and 3N HCl.

Yield Value

Calcium yield value was determined by calculating with the written formula below:

$$\text{Yield value (\%)} = \frac{\text{Dried extracted calcium (g)}}{\text{H. opuntia crude powder (g)}} \times 100\%$$

Calcium content

Calcium content was determined by an Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer Pinaacle 900T, USA) following the method of AOAC (1988). Samples were initially destructed and diluted. Known standard solvent concentrations and samples were read in the AAS subsequently.

Proximate Analysis

Proximate analysis for moisture, ash, fat, and protein was done by following the method of AOAC (2005). Carbohydrate content was calculated using the difference method by adding the total protein, fat,

moisture, and ash content; then, the total amount will be subtracted by 100.

Color

Color measurement was performed by CIELAB color space using the following equation:

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Where ΔL^* stands for the difference between the sample and the standard's lightness, Δa^* for the difference between the sample and the standard's redness (+) or greenness (-), and Δb^* is for the sample and standard's blueness (-) or yellowness (+). The white standard followed the values Wijayanti et al. (2021) mentioned: 93.63 for L^* , -0.94 for a^* , and 0.40 for b^* .

Calcium's whiteness index was also calculated by Zareian et al. (2019) below:

$$WI (\%) = 100 - \sqrt{(100 - L^*)^2 + (a^{*2} + b^{*2})}$$

L^* is for the sample's lightness, a^* is for the sample's redness or greenness, and b^* is for the sample's blueness or yellowness.

Scanning Electron Microscope (SEM)

Analysis regarding the morphology of calcium was done using SEM (Hitachi FlexSEM 1000, Japan) following per method of Wijayanti et al. (2020). Samples prepared and coated with gold were then analyzed for grayscale visualization. The magnification used was 10000 x with 20 kV of accelerating voltage.

Particle Size

Calcium particle size analysis was done using a laser particle size analyzer (Labtron LLPA-C10, UK)

following Wijayanti et al. (2021). Samples were initially dispersed using water and then read with the size measuring range of 0.01 μm until 2000 μm .

X-Ray Diffraction (XRD)

X-Ray Diffraction (Bruker D2 Phaser, USA) was used to analyze calcium obtained from *H. opuntia* following Kusumawati et al. (2022) with slight modification on the angle used. The samples were analyzed at the angle of 2θ 10° to 90°. The wavelength used was 1,54060 Å using the radiation of Cu Ka at step size 0.02°.

Statistical Analysis

Parametric data were analyzed by One-way analysis of variance (ANOVA) after triplication in the experiment. Tukey post-hoc test was performed for sample means comparisons at $p < 0.05$.

Results and Discussion

Yield, calcium, proximate content, color characteristics and particle size of *H. opuntia* extracted using different HCl concentrations are presented in Table 1. Different concentrations of HCl had different impacts on ash, carbohydrate content, L^* , b^* , ΔE -value and Whiteness index ($P < 0.05$). However, no difference in yield, calcium, moisture, fat, protein content, a^* -value and particle size ($P > 0.05$) were observed among treatments.

Yield Value

The yield value of *H. opuntia*-extracted calcium is presented in Table 1. No significant difference among samples was observed when different HCl concentrations were used ($p > 0.05$) since the extraction had been undergone optimally. After passing the optimal

Table 1. Yield, calcium, proximate content, color characteristics and particle size of *H. opuntia* Extracted using different HCl concentrations

Parameters	Samples			
	RM	CH1	CH2	CH3
Yield value (%)	-	28.32±0.38 ^a	29.37±0.80 ^a	27.76±0.76 ^a
Calcium (%) [*]	27.17±0.87	34.97±0.35 ^a	33.80±0.78 ^a	33.90±0.77 ^a
Moisture (%)	2.29±0.29	2.95±0.05 ^a	3.19±0.34 ^a	3.44±0.34 ^a
Ash (%) [*]	87.17±0.80	91.95±0.47 ^b	91.54±0.17 ^b	90.20±0.24 ^a
Fat (%) [*]	0.27±0.05	0.17±0.06 ^a	0.27±0.05 ^a	0.24±0.05 ^a
Protein (%) [*]	3.11±0.01	0.61±0.08 ^a	0.52±0.01 ^a	0.53±0.01 ^a
Carbohydrate (%) [*]	9.44±0.76	7.26±0.57 ^a	7.65±0.18 ^a	9.02±0.27 ^b
L^*	-	86.21±0.83 ^b	86.09±0.60 ^b	82.36±0.65 ^a
a^*	-	0.65±0.03 ^a	0.66±0.11 ^a	0.69±0.02 ^a
b^*	-	8.81±0.02 ^a	9.59±0.04 ^b	12.06±0.08 ^c
ΔE	-	11.33±0.54 ^a	12.00±0.34 ^a	16.30±0.51 ^b
WI (%)	-	83.62±0.70 ^b	83.09±0.47 ^b	78.62±0.58 ^a
Particle size (μm)	-	20.90±0.69 ^a	21.39±0.52 ^a	21.70±0.95 ^a

Notes: Values result from triplicate means \pm SD ($n=3$). Different superscripts in the same row represent significant differences ($p < 0.05$). (RM) raw material, (CH1) calcium extracted using 1N HCl, (CH2) calcium extracted using 2N HCl, (CH3) calcium extracted using 3N HCl. ^{*}Dry weight basis. (-) No analysis was done.

point, the yield value usually experiences a decrease. This statement is in line with Yao et al. (2022), who got a higher yield value after increasing the concentration of acid used in calcium extraction. Still, after passing the optimal point, the yield value would decline. The declining yield value could happen because of the compounds lost during extraction. The too-high acid concentration is the other cause of decreasing yield value after optimal concentration. Mulyani et al. (2021) reported that high-concentrated HCl's ability to extract a compound from a material will be stronger. Therefore, the reaction between compounds and solvent will be balanced, resulting in the higher dissolved compounds. The yield value of calcium in this research was higher than the tilapia (*Oreochromis niloticus*) fishbone extracted repeatedly by Lekahena et al. (2014), which showed a value of 4.41%. Rosnah et al. (2021) reported higher chicken eggshell calcium than this research. However, Herlina et al. (2021) showed lower blood cockle (*Anadara* sp.) calcium yield due to calcination. The calcium yield values were 80% and 1.4%, respectively.

Calcium Content

The calcium content extracted using different HCl concentrations (Table 1) showed no significant difference ($p > 0.05$). The rising calcium content after extraction by immersion occurred due to the ability of HCl to remove protein. Hence, the calcium was concentrated. Herlina et al. (2021) reported that the material immersion in HCl could cause a reaction. As a result, the calcium contained in the material would be dissolved. Premarathna et al. (2022) compared the calcium content among some green, brown, and red algae species and exhibited that green algae had the highest calcium content. The species which contained the highest calcium content in the previously mentioned algae were consecutively *H. opuntia*, *Padina antillarum*, and *Gelidiopsis variabilis*. Their calcium content was 21.28%, 20.21%, and 18.3%, respectively. Another research by Lekahena et al. (2014) showed lower calcium content in tilapia fishbone, which was 21.48%.

Proximate Analysis

Proximate content, including moisture, ash, fat, protein, and carbohydrate content, is displayed in Table 1. The different HCl concentrations used in *H. opuntia* calcium extraction had no impact ($p > 0.05$) on moisture content. The increasing moisture content compared to RM was caused by the reaction between CaCO_3 and HCl that produced water. Rosnah et al. (2021) reported that lower HCl concentrations produced lower moisture content in calcium extracted from eggshells.

The influence of HCl usage in calcium extraction was explained by Habte et al. (2019), water is one of the compounds produced because of the reaction between HCl and CaCO_3 . The moisture content obtained in this research was much lower than that in fresh *H. opuntia* analyzed by Premarathna et al. (2022), which was 95%. The moisture content of Asian sea bass (*Lates calcalifer*) obtained by autoclaving and bleaching by Wijayanti et al. (2020) showed a similar value of 3-4%.

HCl concentration had a significant effect ($p < 0.05$) on the ash content of extracted *H. opuntia* calcium. However, there was no difference in ash content between the CH1 and CH2 samples ($p > 0.05$), yet their value was higher than the CH3 sample ($p < 0.05$). However, this ash content was not linear with calcium content (Table 1), which showed no difference among samples. Even ash content decreased in CH3, but calcium content was similar. The decrease of ash content in CH3 might be due to the decrease of non-calcium-mineral, which was dissolved and removed during extraction. Soltani et al. (2019) explained that leaching could dissolve apatite contained in a material because calcium has high solubility in acid solvents. Lowering ash content in CH3 was caused by the HCl concentration that was too strong (Rahayu et al. 2022). The increasing ash content after extraction (CH1, CH2, and CH3) was noticed due to the lowering protein content (Table 1). Kusumawati et al. (2022) explained that increased ash content after chemical extraction decreased protein. The ash content in this research is higher than the ash contained in fresh *H. opuntia* reported by Premarathna et al. (2022) was 47.36%.

The fat contained in the calcium extracted from *H. opuntia* showed no significant difference ($p > 0.05$) among all samples (CH1, CH2, and CH3). No difference in fat content was observed among all samples and RM, which already had a low-fat content (0.27%). Based on the analysis, it had been known that the fat contained in *H. opuntia* was already shallow. No change in the fat content of *H. opuntia* after calcium extraction showed that HCl in this study couldn't remove the fat content of samples. Regarding the analysis of fats in algae, Afonso et al. (2021) also reported that algae had a low amount of fats. The fat in this research was higher than Premarathna et al. (2022), which reported 0.036% fat contained in fresh *H. opuntia*, yet still lower compared to Asian sea bass bio-calcium fat content, which shows a range value of 10-12% (Wijayanti et al. 2020).

No difference in protein content among all samples (CH1, CH2, and CH3) was observed ($p > 0.05$). Thus, it showed that HCl concentration did not affect the protein content of extracted *H. opuntia* calcium.

However, the protein content sharply decreased (in comparison with RM) after extraction using HCl. The lowering protein content in CH1, CH2, and CH3 correlated with using HCl as the solvent in extraction. Based on the pH measurement conducted during the research, the pH showed 2. An extremely acidic environment can cause decreased protein, leading to lower protein content in this research. Zhang and Ertbjerg (2019) experienced protein denaturation at a pH lower than 5.2. Applying HCl in chemical extraction is known to be the best agent to produce a high deproteinization rate. Hajhosseini et al. (2023) reported that the use of HCl as the agent for deproteinization showed the highest number rather than the deproteinization rate number using trichloroacetic acid (TCA). Premarathna et al. (2022) reported higher protein content in fresh *H. opuntia* compared to this research, which was 16.72%.

The concentration of HCl during extraction had a different effect on carbohydrate content ($P < 0.05$). The highest carbohydrate content was noticed for CH3, the highest concentration of HCl used in extraction. However, no significant difference in carbohydrate content ($p > 0.05$) was found between CH1 and CH2. The declining value of carbohydrate content in CH1 and CH2 was caused by HCl's ability to remove polysaccharide compounds that sorted out carbohydrates. Huang et al. (2021) explained that the ability of HCl to reduce polysaccharides in a material was higher than TCA. The carbohydrates contained within can impact the appearance of a product, for instance, color (Sumarto et al., 2021). The carbohydrate value of extracted *H. opuntia* calcium obtained in this research was still higher than that of Raja et al. (2020), which showed a value of 3.24% in the dried *H. opuntia*.

Color

The color characteristics of *H. opuntia* calcium powders are shown in Table 1. Different HCl concentrations used in the *H. opuntia* calcium extraction had different effects on lightness (L^*), yellowness/blueness (b^*), DE*-value and whiteness index (WI) ($P < 0.05\%$). However, different concentrations of HCl had no impact on the redness/greenness (a^*) of calcium extracted from *H. opuntia* ($P > 0.05$). The lowest L^* -value was noticed for the CH3 sample ($P < 0.05$). However, no lightness difference was found between CH1 and CH2 ($P < 0.05$). The decreasing L^* -value in CH3 might be related to the increasing carbohydrate value in CH3 shown in Table 1. Lekahena et al. (2014) stated that melamine formation, which led to the decreasing L^* -value, could be caused by the reaction between the acid used in

extraction and the aldehyde contained in carbohydrates. The value of L^* in this study was lower than the Asian sea bass bio-calcium's L^* -value obtained by Wijayanti et al. (2020), which was around 90 due to the bleaching agent used in that study. Using bleaching agents like hydrogen peroxide and sodium hypochlorite could rise the L^* -value in Asian sea bass bio-calcium (Wijayanti et al. 2020).

The use of different HCl concentrations had no impact on the redness or greenness (a^*) value of all samples ($p > 0.05$). The a^* -value in this research showed positive (+) values as the meaning of the redness tendency. The same a^* -value in this study was related to carbonate content in calcium powder. A high carbonate level contained in the sample would result in a lower a^* -value. Sun et al. (2011) mentioned that the carbonate content in a sample affected its a^* -value, while organic compounds did not have any strong relation with a^* -value. Liang et al. (2023) added that organic compounds were not related to a^* -value since the color produced tended to be darker and not red. The value of a^* in this study was higher than the a^* -value obtained by Wijayanti et al. (2020) in Asian sea bass bio-calcium because of the use of hydrogen peroxide and sodium hypochlorite.

HCl concentrations significantly impacted the b^* -value of calcium extracted from *H. opuntia* ($P < 0.05$). The differences in b^* -value among all samples (CH1, CH2 and CH3) were noticed ($P < 0.05$). The highest b^* -value was observed for CH3, while the lowest b^* -value was noticed for CH1 ($P < 0.05$). In the highest concentrations of HCl, the highest b^* -value was noticed. The positive (+) symbol on b^* values means that CH1, CH2, and CH3 had a yellowness tendency. The increasing b^* -value was caused by the impurity that had not dissolved during extraction. Kemperl and Macek (2009) mentioned that the high yellowness value in CaCO_3 powder was caused by the undissolved impurities concentrated in precipitated calcium powder. Wijayanti et al. (2020) also obtained an increasing Asian sea bass bio-calcium's b^* -value, ranging from 5 to 10 because of the Maillard reaction. The reaction happened due to the reaction between free amino acids and carbonyl due to fat oxidation during bleaching.

Difference concentrations significantly affected the ΔE -value of *H. opuntia* calcium ($P < 0.05$). The highest value of ΔE was found for CH3 ($P < 0.05$), while the lowest ΔE -value was noticed for CH1 sample ($P < 0.05$). The higher ΔE obtained in a sample referred to the bigger difference between the sample and the white standard. The ΔE -value in all samples (11-16) showed that they were similar to the white standard. Yildirim et al. (2020) added that the ΔE -values are categorized into < 1 for the unseen difference between

sample and standard, 1-2 for the difference between sample and standard that is slightly seen after deep observation, 2-10 for the difference between sample and standard that is slightly seen without deep observation, 11-49 for the similar color between sample and standard, and 100 for completely different sample and standard. Wijayanti et al. (2020) obtained lower ΔE -values in Asian sea bass bio-calcium, which ranged from 5 to 10 and stated that the lower ΔE obtained, the brighter the bio-calcium powder appeared.

Different HCl concentrations used in *H. opuntia* calcium extraction had a significant impact on the whiteness index (WI) ($p < 0.05$). The value of WI in CH3 was the lowest, meaning that 3N of HCl affected on sample's WI ($p < 0.05$). The value of WI in this study was inversely proportional to b^* -value (Table 1). Hence, the sample with the lowest WI value showed the highest b^* -value. The high yellowness is resulting in a low WI. This statement was in line with Varan and Altay (2022) that yellowness will affect a sample's whiteness. The visual appearance among samples was also different, especially in CH3, which had a more yellowish color (Figure 1). Kusumawati et al. (2022) explained that the physical extraction method had lower bioavailability than chemical extraction, such as acidic and alkaline. Another factor that could lead to low WI value is undissolved impurities. Sulistiyono et al. (2018) exhibited that commercial CaCO_3 had higher WI than non-commercial CaCO_3 extracted by HCl because of the non-calcium mineral that did not dissolve during extraction. Wijayanti et al. (2020) mentioned that using

bleaching agents could affect the WI of the Asian sea bass bio-calcium. The WI value in this study was lower than CaCO_3 obtained by Erdogan and Eken (2017), which exhibited a value of 91,28% because of the calcination using very high temperatures.

Particle Size

No significant difference ($p > 0.05$) was observed in the particle size of calcium powder obtained from *H. opuntia* as affected by varying concentrations of HCl (Table 1). Thus, using different HCl concentrations did not influence the size of extracted *H. opuntia* calcium ($p > 0.05$). Ray et al. (2021) stated the opposite, where the particle size experienced a reduction in line with the increasing concentration of solvent used in extraction. Wijayanti et al. (2020) proved pre-treatment before the extraction process, such as applying pressure heating, to produce finer bio-calcium powder texture and more uniform particle size. In the other study, Wijayanti et al. (2021) stated that using ultrasonic waves through ultrasonication was also proven to produce four to nine times smaller bio-calcium particle size.

The particle size distribution curve of calcium extracted from *H. opuntia* using different concentrations of HCl is shown in Figure 1. A Monomodal (one peak) pattern was observed in all samples, and a slight difference in the size of the curves was observed. CH1 showed the narrowest shape, while CH3 showed the widest. The curve narrowness in CH1

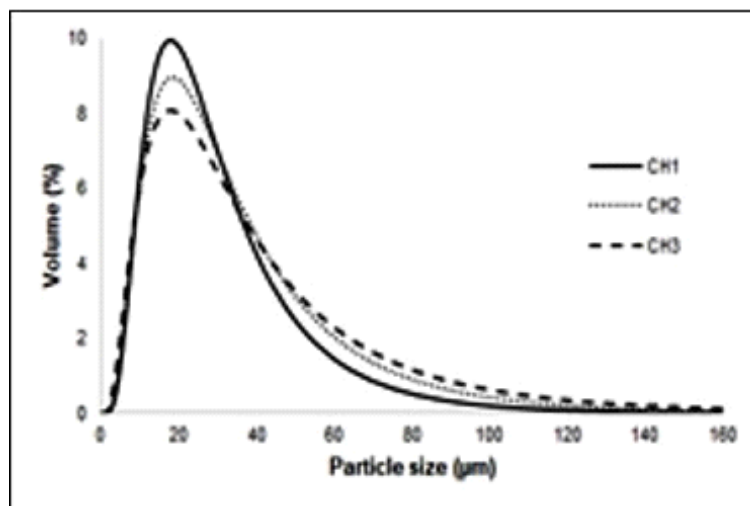


Figure 1. Particle Size Distribution of Extracted Calcium from *H. opuntia*. (CH1) calcium extracted using 1N HCl, (CH2) calcium extracted using 2N HCl, (CH3) calcium extracted using 3N HCl.

could be referred to as the more homogenous calcium particle shown in the SEM imaging (Figure 1). The wideness of the curves implied the homogeneity of the material's particle size. The narrower it gets, the

more homogenous the particle size. The amount of the peak that appeared could also be used as the standard to determine the homogeneity of the particle size. Wijayanti et al. (2020) exhibited two different types of

curves, which were monomodal (one peak) and bimodal (two peaks), where the monomodal curve implies better homogeneity of particle size.

Scanning Electron Microscope (SEM)

The microstructure of *H. opuntia* calcium powder extracted using different HCl concentrations is displayed in Figure 2. (Figure 2a, 2b, and 2c). Different microstructures of CH1, CH2 and CH3 samples were observed. CH1 showed a single cubical shape particle, which was also non-porous. For the CH2 sample, agglomeration started occurring in some parts of the particle and still showed a non-porous surface. The significant change looked very differently in CH3, which completely agglomerated and the shape was

more rounded. These statements proved that different HCl concentrations affected the morphology of calcium extracted from *H. opuntia*. This result was in line with Ray et al. (2021), which showed the CaCO₃ particles were non-porous, and the shape became rounder as the extraction solvent concentration raised. The cause of the particle's agglomeration was because of the exceeded amount of Ca²⁺ in the solvent that led to the alteration of ionic charge that became neutral.

X-Ray Diffraction (XRD)

Diffraction patterns of calcium extracted from *H. opuntia* using HCl at different concentrations are shown in Figure 3. Scanning at an angle of 2θ 10° to 90° showed no difference in diffraction pattern

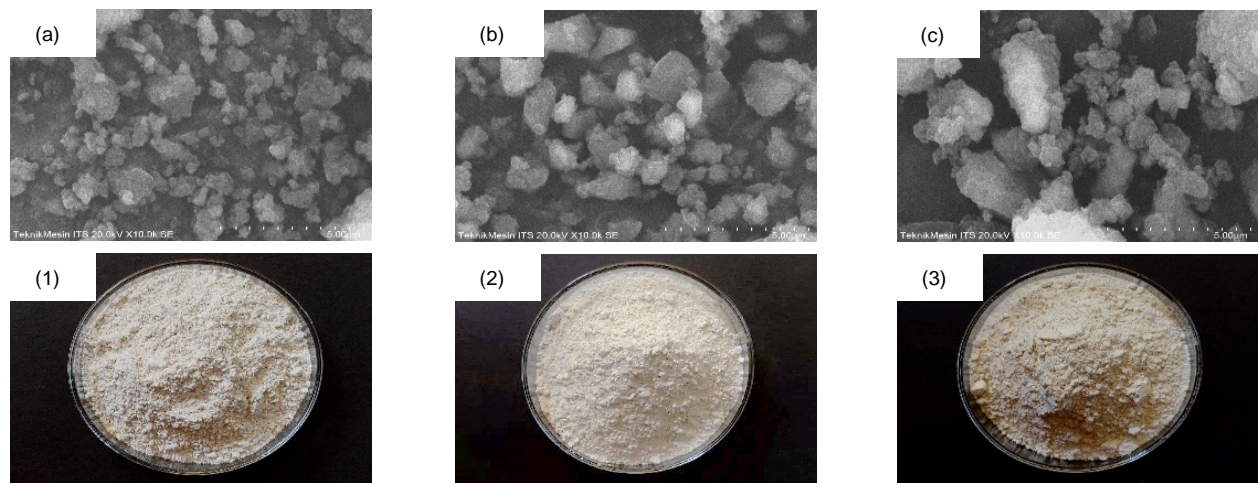


Figure 2. Microstructure (a, b, c) and the powder (1, 2, 3) of Calcium from *H. opuntia* extracted using 1N HCl (a, 1); 2N HCl (b, 2) and 3N HCl (c, 3).

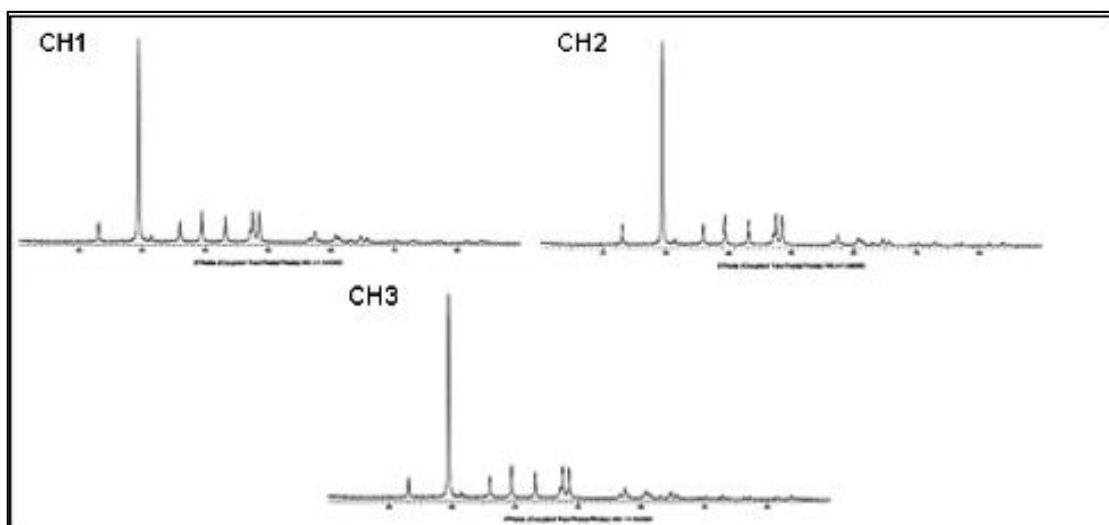


Figure 3. *H. opuntia* Extracted Calcium Diffraction Pattern. (CH1) calcium extracted using 1N HCl, (CH2) calcium extracted using 2N HCl, (CH3) calcium extracted using 3N HCl.

among all samples. Three diffractogram patterns showed that using different concentrations of HCl during extraction produced calcium with the same phase, calcite. The degree of crystallinity for CH1, CH2, and CH3 was respectively 79.4%, 80.2%, and 78%. Based on the analysis by comparing the diffraction pattern with the database, it clearly understood that the calcium extracted from *H. opuntia* had the same pattern as CaCO_3 . This result was connected with the statement of Swarnam et al. (2021) that *H. opuntia* contains a high level of CaCO_3 within cells. The diffractogram pattern in this research was also the same as eggshells and seashells' which were exhibited by Supriyanto et al. (2019) and Dampang et al. (2019). However, the pattern of calcium extracted from *H. opuntia* differed from that extracted from Asian sea bass bone carried out by Wijayanti et al. (2020), as the pattern referred to hydroxyapatite.

The number of peaks that appeared in CH1 was 28, with the highest peak appearance at 29.4° , 39.4° , 43.1° , 47.5° and 48.5° . CH2 showed 25 peaks with the highest appearance at the angle of 29.4° , 39.4° , 43.1° , 47.5° and 48.4° . 24 peaks appeared in the CH3 diffractogram pattern, with the highest peak appearance at the angle of 29.4° , 39.4° , 43.2° , 47.5° , and 48.5° . The number of angles that appeared also strengthened the proof that different HCl concentrations used did not affect the pattern of calcium extracted from *H. opuntia*. The consistency of peak appearance in this research was similar to Luo et al. (2020), which showed similar highest peaks that emerged at the angle of 29.4° , 36° , 39.4° , 43.2° , 47.5° , and 48.5° .

Conclusion

The use of different hydrochloric acid (HCl) concentrations gave a significant effect ($p < 0.05$) on ash content, carbohydrate content, lightness (L^*), yellowness or blueness (b^*), ΔE -value, whiteness index (WI), but no difference effect on calcium, moisture, protein, fat content and a^* -value were observed when different HCL's concentrations were used. The calcium extracted by HCl 1N (CH1) showed the best result for color characteristics. The SEM microstructural analysis showed a non-porous surface and a single cubical shape in CH1. The diffractogram pattern of calcium extracted from *H. opuntia* using different concentrations by XRD exhibited the same pattern as CaCO_3 for all samples. HCl solvent at 1 N can produce calcium powder from *H. opuntia* with high calcium content and better color characteristics.

Supplementary Materials

Supplementary materials is not available for this article

References

- Afonso, C., Correia, A.P., Freitas, M.V., Baptista, T., Neves, M., & Mouga, T. (2021). Seasonal Changes in the Nutritional Composition of *Agarophyton vermiculophyllum* (Rhodopyta, Gracilariales) from the Center of Portuga. *Foods*, 10, 2-17. <https://doi.org/10.3390/foods10051145>.
- Agustini, T.W., A.S. Fahmi, I. Widowati dan A. Sarwono. (2011). Pemanfaatan Limbah Cangkang Kerang Simpson (Amusium pleuronectes) dalam Pembuatan Cookies Kaya Kalsium. *Jurnal Pengolahan Hasil Perikanan Indonesia*, 14(1), 8-13.
- AOAC. (1988). Official Methods of Analysis of Association of Chemistry. Association of Official Analytical Chemists. Washington DC.
- AOAC. (2005). Official Methods of Analysis of Association of Chemistry. Association of Official Analytical Chemists. Washington DC.
- Asih, T., Khayuridlo, M., Noor, R., & Muhfahroyin. (2019). Biodiversity and Potential Use of Macro Algae in Pesisir Barat Lampung. *Biosaintifika: Journal of Biology & Biology Education*, 11(1), 100-107. <http://dx.doi.org/10.15294/biosaintifika.v11i1.16532>.
- Benjakul, S., Mad-Ali, S. & Sookchoo, P. (2017). Characteristics of Biocalcium Powders from Pre-Cooked Tongol (*Thunnus tonggol*) And Yellowfin (*Thunnus albacores*) Tuna Bones. *Food Biophysics*, 12, 412-421.
- Dampang, S., Purwanti, E., Destyorini, F., Kurniawan, S.B., Abdullah, S.R.S., & Imron, M.F. (2021). Analysis of Optimum Temperature and Calcination Time in the Production of CaO Using Sea Shells Waste as CaCO_3 Source. *Journal of Ecological Engineering*, 22(5), 221-228. <https://doi.org/10.12911/22998993/135316>.
- Erdogan, N., & Eken, H.A. (2017). Precipitated Calcium Carbonate Production, Synthesis and Properties. *Physicochemical Problems of Mineral Processing*, 53(1), 57-68. <http://dx.doi.org/10.5277/ppmp170105>.
- Habte, L., Shiferaw, N., Mulatu, D., Thenepalli, T., Chilakala, R., & Ahn, J.W. (2019). Synthesis of Nano-Calcium Oxide from Waste Eggshell by Sol-Gel Method. *Sustainability*, 11, 1-10. <http://dx.doi.org/10.3390/su11113196>.
- Hajhosseini, A., Sharifan, A., Eftekhari, Z., Alavi, A., & Doroud, D. (2023). Optimal Extraction and Deproteinization Method for Mannoprotein Purification from *Kluyveromyces marxianus*. *Iranian Biomedical Journal*, 27(4), 100-106. <http://dx.doi.org/10.52547/ibj.3200>.
- Herlina, D.S., Sulmartiwi, L., & Masithah, E.D. (2021). Effect of Soaking Time of Blood Cockle (*Anadara* sp.) Shells Powder with Hydrochloric Acid on the Characteristics of Nano Calcium. *IOP Conf. Series: Earth and Environmental Science*, 679, 1-8. <https://doi.org/10.1088/1755-1315/679/1/012050>.
- Huang, G., Chen, F., Yang, W., & Huang, H. (2021). Preparation, Deproteinization and Comparison of Bioactive Polysaccharides. *Trends in Food Science & Technology*, 109, 564-568. <https://doi.org/10.1016/j.tifs.2021.01.038>.
- Idowu, A. T., Benjakul, S., Sinthusamran, S., Sae-leaw, T., Suzuki, N., Kitani, Y. & Sookchoo, P. (2020). Effect of Alkaline Treatment on Characteristics of Bio-Calcium and

- Hydroxyapatite Powders Derived from Salmon Bone. *Applied Sciences*, 10, 4141.
- Kim, D.C., Won, S.I. & In, M.J. (2017). Combination Effect of Acetic Acid and Citric Acid on Calcium and Phosphorus Extraction from Shank Bone. *Journal of Applied Biological Chemistry*, 60(1), 19-22.
- Kemperl, J., & Macek, J. (2009). Precipitation of Calcium Carbonate from Hydrated Lime of Variable Reactivity, Granulation and Optical Properties. *International Journal of Mineral Processing*, 93, 84-88. <http://dx.doi.org/10.1016/j.minpro.2009.05.006>
- Kepel, R.C., Lumingas, L.J.L., Tombokan J.L., & Mantiri, D.M.H. (2021). Biomineral Characterization and Phytochemical Profile of Green Algae *Halimeda macroloba* and *Halimeda opuntia* from Coastal Waters of Tanjung Merah, Bitung City, North Sulawesi, Indonesia. *AACL Bioflux*, 14(6), 3217-3230.
- Kurkinen, S., Virolainen, S., & Sainio, T. (2021). Recovery of Rare Earth Elements from Phosphogypsum Waste in Resin-Leach Process by Eluting with Biodegradable Complexing Agents. *Hydrometallurgy*, 201, 1-9. <https://doi.org/10.1016/j.hydromet.2021.105569>.
- Kusumawati, P., Triwitono, P., Anggrahini, S., & Pranoto, Y. (2022). Nano-Calcium Powder Properties from Six Commercial Fish Bone Waste in Indonesia. *Squalen Bulletin*, 17(1), 1-12. <https://doi.org/10.15578/squalen.601>.
- Lekahena, V., Faridah, D.N., Syarif, R., & Peranginangin, R. (2014). Karakterisasi Fisikakimia Nanokalsium Hasil Ekstraksi Tulang Ikan Nila Menggunakan Larutan Basa dan Asam. *Jurnal Teknologi dan Industri Pangan*, 25(1), 57-64. <http://dx.doi.org/10.6066/jtip.2014.25.1.57>.
- Lekahena, V.N.J., Saniapon, J., Kamisi, H.L., & Tupamahu, Y.M. (2019). Physicochemical Characteristic of Alkali treated Cottonii (ATC) Flour of *Kappaphycus alvarezii* Seaweed from Morotai Island in North Maluku. *Journal of Physics: Conference Series*, 1364, 1-8. <https://doi.org/10.1088/1742-6596/1364/1/012011>.
- Liang, X., Wang, X., Zhai, X., Niu, Q., & Li, J. (2023). Color Changes in Yardang Strata Sediment in the Dunhuang Yardang National Geopark, Northwest China: Controlling Factors and Significance for Sedimentary Environments. *Frontiers in Earth Science*, 10, 1-15. <https://doi.org/10.3389/feart.2022.1107213>.
- Luo, X., Song, X., Cao, Y., Song, L., & Bu, X. (2020). Investigation of Calcium Carbonate Synthesized by *Steamed Ammonia Liquid Waste Without Use of Additives*. *Royal Society of Chemistry Advances*, 10, 7976-7986. <https://doi.org/10.1039/c9ra10460g>.
- Mulyani, S., Rohmeita, D., & Legowo, A.M. (2021). Karakteristik Kalsium dari Tulang Ikan Bandeng (*Chanos chanos*) yang Diekstraksi Menggunakan Larutan HCl. *Journal of Nutrition College*, 10(4), 321-327.
- Premarathna, A.D., Tuvikene, R., Fernando, P.H.P., Adhikari, R., Perea, M.C.N., Ranahewa, T.H., Howlander, M.M., Wangchuk P., Jayasooriya, A.P., & Rajapakse, R.P.V.J. (2022). Comparative Analysis of Proximate Compositions, Mineral and Functional Chemical Groups of 15 Different Seaweed Species. *Scientific Reports*, 12, 1-13. <https://doi.org/10.1038/s41598-022-23609-8>.
- Rahayu, A.P., Islami, A.F., Saputra, E., Sulmartiwi, L., Rahmah, A.U., & Kurnia, K.A. (2022). The Impact of the Different Types of Acid Solution on the Extraction and Adsorption Performance of Chitin from Shrimp Shell Waste. *International Journal of Biological Macromolecules*, 194, 843-850. <https://doi.org/10.1016/j.ijbiomac.2021.11.137>.
- Raja, R., Hemaiswarya, S., Sridhar, S., Algarsamy, A., Ganesa, V., Elumalai, S., & Carvalho, I.S. (2020). Evaluation of Proximate Composition, Antioxidant Properties, and Phylogenetic Analysis of Two Edible Seaweeds. *Smart Science*, 1-6. <https://doi.org/10.1080/23080477.2020.1795338>.
- Ray, S., Bhattacharya, T.K., Singh, V.K., Deb, D., Ghosh, S., & Das, S. (2021). Non-Isothermal Decomposition Kinetics of Nano-Scale CaCO₃ as a Function of Particle Size Variation. *Ceramics International*, 47, 858-864. <https://doi.org/10.1016/j.ceramint.2020.08.198>.
- Rosnah, Taslim, N.A., Aman, A.M., Idris, I., As'ad, S., Bukhari, A., & Wahyudin, E. (2021). Physicochemical Characteristics of Chicken Eggshell Flour Produced by Hydrochloric Acid and Acetic Acid Extraction. *Open Access Macedonian Journal of Medical Sciences*, 9(A), 428-432. <https://doi.org/10.3889/oamjms.2021.6238>.
- Soltani, F., Abdollahy, M., Petersen, J., Ram, R., Koleini, S.M.J., & Moradkhani, D. (2019). Leaching and Recovery of Phosphate and Rare Earth Elements from an Iron-Rich Fluorapatite Concentrate: Part II: Selective Leaching of Calcium and Phosphate and Acid Baking of the Residue. *Hydrometallurgy*, 184, 29-38. <https://doi.org/10.1016/j.hydromet.2018.12.024>.
- Sulistiyono, E., Handayani, M., Firdiyono, F., & Fajarani, E.N. (2018). Precipitation Rate Investigation on Synthesis of Precipitate Calcium Carbonate. *IOP Conf. Series: Materials Sciences and Engineering*, 333, 1-5. <https://doi.org/10.1088/1757-899X/333/1/012042>.
- Sumarto, Desmelati, Sari, N.I., Anggraini, R.M., & Arieska, L. (2021). Characteristic of Nano-Calcium Bone from a Different Species of Catfish (*Pangasius hypophthalmus*, *Clarias batrachus*, *Hemibagrus nemurus*, and *Paraplotosus albilabris*). *IOP Conf. Series: Earth and Environmental Science*, 965, 1-8. <https://doi.org/10.1088/1755-1315/695/1/012055>.
- Sun, Y., He, L., Liang, L., & An, Z. (2011). Changing Color of Chinese Loess: Geochemical Constraint and Paleoclimatic Significance. *Journal of Asian Earth Sciences*, 40, 1131-1138. <https://doi.org/10.1016/j.jseas.2020.08.006>.
- Sunardi & Krismawati, E.D. (2021). Pengaruh Waktu Ekstraksi dan Konsentrasi HCl terhadap Rendemen dan Kadar Kalsium pada Sintesis Nanokalsium Oksida dari Cangkang Telur Ayam. *Seminar Nasional Penelitian dan Pengabdian kepada Masyarakat (SNPPKM)*, 6 Oktober 2021, 605-612.
- Sunardi, Krismawati, E.D., & Mahayana, A. (2020). Sintesis dan Karakterisasi Nanokalsium Oksida dari Cangkang Telur. *ALCHEMY Jurnal Penelitian Kimia*, 16(2), 250-259. <https://doi.org/10.20961/alchemy.16.2.40527.250-259>.
- Supriyanto, Sukarni, N.S.W., Puspitasari, P., & Permanasari, A.A. (2019). Synthesis and Characterization of CaO/CaCO₃ from Quail Eggshell Waste by Solid State Reaction Process.

- AIP Conference Proceedings, 2120, 1-6. <https://doi.org/10.1063/1.5115670>.
- Swarnam, T.P., Velmurugan, A., Shajeeda, V.B., & Kavita, G. (2021). Exploring the Potential of Seaweeds of South Andaman Coast for Agricultural Use. *Journal of the Andaman Science Association*, 26(1), 1-7.
- Varan, N.Y. & Altay, P. (2022). Antibacterial Activity and Thermophysiological Comfort Enhancement by Calcium Chloride Solutions of Highly Elastane Polyamide 66 Fabrics. *Fibers and Polymers*, 23(4), 967-976. <https://doi.org/10.1007/s12221-022-4274-1>.
- Wijayanti, I., Benjakul, S., & Sookchoo, P. (2020). Preheat-Treatment and Bleaching Agents Affect Characteristics of Bio-Calcium from Asian Sea Bass (*Lates calcarifer*) Backbone. *Waste and Biomass Valorization*, 12, 3371-3382. <https://doi.org/10.1007/s12649-020-01224-w>.
- Wijayanti, I., Sookchoo, P., Prodpran, T., Mohan, C.O., Aluko, R.E., & Benjakul, S. (2021). Physical and Chemical Characteristic of Asian Sea Bass Bio-Calcium Powders as Affected by Ultrasonication Treatment and Drying Method. *Journal of Food Biochemistry*, 45(4), e13652. <https://doi.org/10.1002/aocs.12649>.
- Wijayanti, I., Prodpran, T., Sookchoo, P., Nirmal, N., Zhang, B., Balange, A., & Benjakul, S. (2022). Textural, Rheological and Sensorial Properties of Mayonnaise Fortified with Asian Sea Bass Bio-Calcium. *Journal of The American Oil Chemists' Society*, 1-18. <https://doi.org/10.1111/jfbc.13652>.
- Yao, Y., Shi, Y., An, P., Zhang, R., Wang, Z., Hu, X., & Wan, Y. (2022). Optimization of Preparation of Calcium Propionate from Eggshell by Response Surface Methodology (RSM). *Food Science and Technology*, 42, 1-10. <https://doi.org/10.1590/fst.25322>.
- Yildirim, T.M., Khoramnia, R., Masyk, M., Son, H., Auffarth, G.U., & Mayer, C.S. (2020). Aesthetic of Iris Reconstruction with a Custom-Made Artificial Iris Prosthesis. *PLOS ONE*, 15(8), 1-12. <https://doi.org/10.1371/journal.pone.0237616>.
- Zainol, I., Adenan, N.H., Rahim, N.A., & N.A. Jaafar. (2019). Extraction of Natural Hydroxyapatite from Tilapia Fish Scales Using Alkaline Treatment. *Materials Today: Proceedings*, 16, 1942-1948.
- Zareian, M., Tybussek, T., Silcock, P., Bremer, P., Beauchamp, J., & Bohner, N. (2019). Interrelationship Among Myoglobin Forms, Fat Oxidation and Protein Carbonyl in Minced Pork Packaged under Modified Atmosphere. *Food Packaging and Shelf Life*, 20, 1-8. <https://doi.org/10.1016/j.fpsl.2019.100311>.
- Zhang, Y. & Erbjerg, P. (2019). On the Origin of Thaw Loss: Relationship between Freezing Rate and Protein Denaturation. *Food Chemistry*, 299, 1-8. <https://doi.org/10.1016/j.foodchem.2019.125104>.