

Physical Properties and Sensory Evaluation of Agarwood Leaves Extract Powder Through Enzyme Treatment

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Abstract

Enzymes mostly could act on molecular structures allowing for the generation of specific compounds, which is particularly beneficial in the food industry and flavour is a key determinant of food quality which is influenced by enzymes that can enhance undesirable tastes. This study aims to focusing on enhancing its flavour through bromelain enzymatic treatment and food colourants based on the physical properties. Besides that, agarwood leaves extract was extracted using the decoction method. The untreated agarwood leaves extract was subjected to a water bath at 50°C for 5 minutes and followed by treated agarwood leaves extract was water bath at 50°C for 3 hours to ensure the enzyme operates at peak efficiency, promoting the desired reactions without denaturing the enzyme. The extract was then spray-dried at temperatures ranging from 180°C to 200°C, with a feed rate of 10-20 rpm. Bromelain was used at a concentration of 18%, diluted with 1000 mL of distilled water. The treated powder exhibited improved flavour with reduced bitterness and enhanced sweetness. Sensory evaluation confirmed a preference for the enzyme-treated sample in which formulation 2, the treated with bromelain was preferred for its improved sweetness and appearance. Statistical analysis showed significant differences between treated and untreated formulations in acceptance based on the sensory evaluation which is the superscript letter was denoted as significance value ($p < 0.05$) of sample comparing in column order. The combination of bromelain and colourant resulted in a vibrant, leaf-green powder that not only improved the powder's taste but also its visual appeal.

1. Introduction

Enzymes, functioning as biocatalysts which is provide various opportunities for enhancing food flavours [1]. Enzyme bromelain has been reported to have excellent activity in improving the flavour of fresh meat [2]. Essentially, flavour refers to the sensory experience perceived by taste buds upon consuming a food item [3] and colour influences consumer perception and product quality [4]. Moreover, have reported that bromelain could accelerate the proteolysis of golden pomfret protein, softening texture and enhancing flavour [5]. Besides, converting agarwood leaves into a consumable powder, enhancing their flavour through enzyme treatment, and stabilizing its colour through food colourant, could unlock the leaves potential as a valuable food product.

To create the flavour in this powder, the approach that is suitable to be used is by using a hydrolysis enzyme, which is Bromelain. A group of enzymes called hydrolases, commonly referred to as hydrolysis enzymes, catalyse the disintegration of different biomolecules through a hydrolysis reaction [6]. The product,

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which is flavoured agarwood powder, can be used in various ways such as snack coating, food seasoning or acting as salt substitute.

This study aims to develop agarwood leaf extract powder (ALEP), in focusing on enhancing its flavour through bromelain enzymatic treatment and additional of food colourants based on the physical properties. Also, it's highlighted for sensory evaluation of the agarwood leaves extract powder through enzyme treatment.

However, this approach can help agarwood farmers in regions like Bangladesh overcome challenges related to traditional cultivation methods and low yields [7]. By promoting the utilization of all parts of the agarwood tree, this research could ultimately reduce waste, boost profitability for agarwood farmers, and support the growing demand for functional and natural food products [8].

2. Materials and Methods

2.1 Sample Preparation

The preparation and extraction of agarwood leaves for this project involved several critical steps to ensure the quality and efficiency of the process. Fresh leaves of agarwood were selected and thoroughly washed to remove any dirt or impurities. The leaves were then dried using a smokehouse machine at 80°C for 1 hour and 40 minutes [3].

After the drying process, the leaves were carefully ground using a bowl chopper (Dito Sama K120, France) for 5 minutes to reduce particle size and increase surface area, which significantly improved extraction efficiency. After grinding, an aqueous extraction process was performed, where the powdered leaves were infused with water at 95-100°C for 30 minutes using a water bath and the extracted agarwood leaves sample was dispersed with sterile water which is 10 g/100 mL [10]. The pH was 6 ± 0.5 for extracted agarwood solution before thoroughly the enzyme and colourant treatment.

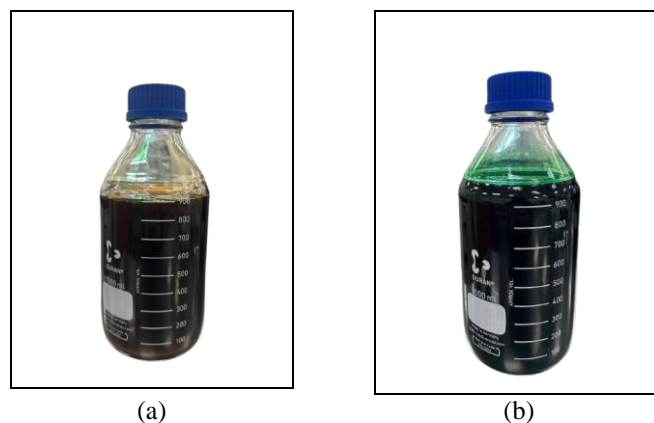


Fig. 1 Agarwood Leaves Extract Solution (a) Untreated Solution; (b) Treated Solution

2.2 Enzyme Treatment

After the aqueous extraction, bromelain enzymes were diluted with distilled water with a concentration of 18% (w/w) and prepared and incubated in a water bath at 50°C for 5 minutes before mixing. After this incubation period, the bromelain solution followed by additional food colourant was combined to the extracted agarwood leaves sample and further incubated at 50°C for 3 hours in a water bath to ensure the enzyme operates at peak efficiency, promoting the desired reactions without denaturing the enzyme. The reaction was terminated by heating the mixture to 90°C for 15 minutes [9]. The use of 18% bromelain for the hydrolysis process of agarwood leaves was found to be optimal for efficient enzymatic activity [10].

2.3 Percentage of Colourant

Food colourant solutions were used in 0.79% white, 0.18% apple green, and 0.18% brown in distilled water by [17] and incubated at 50°C for 5 minutes. After that, the bromelain solution and colourants were combined with the extracted agarwood leaves sample and incubated at 50°C for 3 hours.

2.4 Spray Drying

The agarwood extract went through the spray drying to produce a stable powder. Maltodextrin (18%) was added as a carrier agent as a method outlined by [11] with minor modifications. The mixture was dried at 150-180°C, with a feed flow rate of 10-20 rpm and a 0.7 mm nozzle size.

2.5 Determination of Moisture Content

The moisture content analysis was carried out using the method described by [12]. The moisture content of the Agarwood Leaves extract powder was automatically measured using the moisture analyzer (A&DMX-50, Japan).

2.6 Determination of Dispersibility

Dispersibility was carried out using the method described by [13]. 5 grams of ALEP powder was added to 50 mL of distilled water at 20°C in a 50 mL measuring cylinder. The mixture was stirred vigorously and left undisturbed for 3 hours to allow the solid particles to settle. Afterward, the volume of the liquid phase was measured, and the dispersibility was calculated as the ratio of the liquid phase by using the following formula.

$$\text{Dispersibility (\%)} = \left(\frac{V_{\text{liquid}}}{V_{\text{total}}} \right) 100 \quad (1)$$

2.7 Determination of Colour

The colour of the agarwood leaves extract powder was analysed using a (HunterLab Miniscan EZ 4500L colour spectrophotometer, USA) which measured the light reflected or transmitted by the sample. The device was calibrated with a white reference plate or using standard black and white tiles [14]. The L* (lightness), a* (green/red), and b* (blue/yellow) values were obtained by evenly spreading the sample and taking triplicate readings, with the mean values providing a precise colour analysis of the powder.

2.8 Sensory evaluation

For sensory evaluation, the agarwood leaves extract powder divided into three parts in which one of untreated powder and the two of treated powder for each consumer were taken. The panel for sensory evaluation consisted of 60 untrained panellists (18-25 years old). According to a seven-point hedonic scale (1: extremely dislike to 7: extremely like), the appearance, taste, odor, sweetness, bitterness, colour, and the overall acceptance of powder were evaluated [15].

2.9 Statistical Analysis


Two powders were used in which the untreated and treated agarwood leaves extracted powder. All the experiments were recorded in triplicate. Data were expressed as mean \pm standard deviation which is to compare and evaluated using one-way analysis of variance (ANOVA) followed by Tukey's Significant Differences post hoc test, performed using the SPSS 22.2 version software. A statistical difference at $p < 0.05$ was considered significant.


3. Result and Discussion

3.1 Comparison of Agarwood Leaves Extract Powder on Physical Properties

The result shows two comparisons of untreated and treated agarwood leaves extract powder using statistical analysis to assess significant differences. Data were collected through triplicate tests, and the mean and standard deviation were calculated. ANOVA with a significance level of $p < 0.05$ was used to determine differences between the formulations, with analysis conducted using SPSS 22.2. Also, the optimal conditions of pH 6 and 50°C were found to maximize enzyme activity, ensuring the best possible flavour extraction.

Table 1 Physical properties of agarwood leaves extract powder

Agarwood Leaves Extract Powder	Visual Appearance	Physical Properties				
		Colour index			Dispersibility (%)	Moisture Content (%)
		Lightness (L*)	Red/green (a*)	Yellow/blue (b*)		
Untreated powder		72.557 \pm 0.490 ^a	3.517 \pm 0.481 ^a	14.863 \pm 0.845 ^a	82.67 \pm 4.04 ^a	4.963 \pm 0.866 ^a

Treated powder		65.960 ± 0.255 ^b	3.170 ± 0.118 ^a	12.917 ± 0.476 ^b	83.00 ± 8.19 ^a	3.603 ± 0.760 ^a
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*The result was expressed as mean ± standard deviation for each sample.
 *a/b was denoted as significance value (p<0.05) of sample comparing in column order

Table 1 shows the agarwood leaves extract powder for both untreated and treated samples, highlighting the differences in visual appearance before and after enzyme treatment and colour addition.

The colour analysis of agarwood powder, both untreated and treated with enzyme and colour, revealed significant differences in their colour result. The untreated agarwood powder exhibited a higher lightness (L* = 72.557), with a slight reddish (a* = 3.517) and yellowish (b* = 14.863) tint, consistent with typical light brown hues. In contrast, the treated powder displayed a lower lightness (L* = 65.960), indicating a darker, more consistent greenish tone. The a* value (3.170) slightly decreased, suggesting less redness, while the b* value (12.917) also decreased, reflecting a shift from yellow to green. These findings indicate that the enzyme and colour treatment significantly altered the agarwood powder's colour, shifting it from light brown to leaf green, with the most notable changes in lightness and yellow intensity.

By measuring the liquid phase volume after the mixture had settled for 3 hours, both untreated and treated with enzyme and colour, revealed no significant differences. The results showed that the treated agarwood leaves extract sample has a slightly higher dispersibility (83.00 ± 8.19) compared to the untreated agarwood leaves extract powder (82.67 ± 4.04), indicating a marginal improvement in its ability to disperse in water. These findings suggest that the enzyme and colour treatment did not drastically alter the dispersibility of the powder, but it did show a slight enhancement in dispersion, as observed in similar studies [13].

The moisture content of the agarwood leaves extract powders varied and both untreated and treated with enzyme and colour, revealed no significant differences. The untreated agarwood leaves extract powder exhibiting a higher moisture content 4.963 compared to the treated in which agarwood leaves extract powder with enzyme and colour treatment 3.603. Both values fall within the acceptable range, as the national regulation for seasoning powders requires moisture content to be below 5%, and food-grade salt should have less than 3% moisture [13]. The higher moisture content in the untreated agarwood leaves extract powder resulted in slower dissolution and increased clumping when exposed to air. Despite these differences in physical properties, both powders exhibited distinct sensory characteristics, consistent with variations observed in their original extracts.

3.2 Sensory Evaluation of Agarwood Leaves Extract Powder

Three agarwood leaves extract powder formulations were presented to panellists [16]: Formulation 1, which contained 100 grams of untreated agarwood leaves extract powder, 3 grams of salt, 5 grams of sugar, and 2 grams of sodium benzoate; formulation 2, which consisted of 90 grams of treated agarwood leaves extract powder, 3 grams of salt, 5 grams of sugar, and 2 grams of sodium benzoate; and formulation 3, which included 95 grams of treated agarwood leaves extract powder, 3 grams of salt, 5 grams of sugar, and 1 gram of sodium benzoate. The formulations 2 and 3 were treated with bromelain enzyme, which altered the flavour profile compared to the untreated agarwood leaves extract powder in formulation 1. The panellists' preferences revealed that formulations 1 and 2 were treated with enzyme which differed in taste from the control in which formulation 1, with significant differences observed in sweetness, bitterness and overall flavour acceptance.

Table 2 Sensory evaluation of agarwood leaves extract powder

Sample	Appearance	Colour	Odor	Sweetness	Bitterness	Overall Acceptance
Formulation 1	5.100 ± 1.654 ^a	4.933 ± 1.425 ^a	4.417 ± 1.565 ^a	2.200 ± 1.400 ^a	2.883 ± 2.387 ^a	2.617 ± 1.367 ^b
Formulation 2	5.633 ± 1.353 ^a	5.600 ± 1.265 ^{ab}	5.133 ± 1.443 ^{ab}	5.033 ± 1.262 ^a	4.083 ± 1.788 ^a	4.983 ± 1.359 ^a
Formulation 3	5.383 ± 1.439 ^a	5.283 ± 1.474 ^b	5.033 ± 1.495 ^b	4.783 ± 1.814 ^b	4.117 ± 1.905 ^b	4.839 ± 1.610 ^a

*The result was expressed as mean ± standard deviation for each sample.
 *a/b was denoted as significance value (p<0.05) of sample comparing in column order

The result shown on Table 2 is the sensory evaluation of three agarwood leaves extract powder samples revealing notable differences in their appearance, odor, colour, sweetness, and bitterness. For formulation 1 was the lowest mean scores in which the attributes have no significance difference between formulation 2 and 3 but for attributes colour, odor, sweetness, bitterness and overall acceptance have significant difference between formulation 3. Formulation 2 was the highest mean score and there is no significance difference between formulation 1 and 3 for attributes appearance, colour and odor.

Next, there is significance differences for attributes sweetness, bitterness and overall acceptance between formulation 2 and 3. In formulation 3, for attribute appearance there is no significance difference between formulation 1 and 2 and for colour and odor there is also no significance difference between formulation 2 but for attributes sweetness, bitterness and overall acceptance have no significance differences between formulation 2 and 3. However, it related to previous studies in which the enzymatic hydrolysis also breaks peptide bonds of protein sources using commercial proteases, such as bromelain, papain and alcalase and enzymatic hydrolysate gives meaty and savory flavour which it has to meet the consumer preference which is described by Sonklin [11].

Also, ANOVA test showed significant differences in colour until bitterness, with panellists able to distinguish the varying levels of bitterness in each sample, while no significant differences were found in appearance. The findings suggest that the balance of ingredients, particularly the concentration of agarwood leaves extract powder, plays a key role in the overall acceptance of the powder.

4. Conclusion

In this study, both powders show the significant difference in which the treated agarwood leaves extract powder were able to improve the flavour of agarwood leaves extract powder by using enzyme treatment and food colouring, turning. The use of bromelain enzyme effectively neutralized the natural bitterness, while enhancing the extraction of light sweet flavours from the agarwood. The combination of bromelain and colourant resulted in a vibrant, leaf-green powder that not only improved the powders. For instance, the consumer preference for both taste and visual appeal would likely result in higher demand. Furthermore, conducting sensory testing and market analysis would provide valuable insights into consumer preferences, guiding the development of new product lines in industrial applications. Also, the potential impact on the sensory evaluation attribute is by the interaction of bromelain with other compounds in the extract could lead to off-flavours or undesirable aromas if not carefully controlled. This study demonstrated the effectiveness of enzymatic treatment in improving the taste and appearance of agarwood powder, making it more suitable for a variety of applications and future studies could also focus on making the enzymatic process more environmentally sustainable and investigating the stability of treated powder during storage or exploring other enzymes for flavour enhancement.

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Conflict of Interest

Authors declare that there is no conflict of interest regarding the publication of the paper.

Author Contribution

*The authors confirm contribution to the paper as follows: **study conception and design:** Nur Fathihah Jamiran, Balkis A. Talip; **data collection:** Nur Fathihah Jamiran; **analysis and interpretation of results:** Nur Fathihah Jamiran, Balkis A. Talip; **draft manuscript preparation:** Nur Fathihah Jamiran, Balkis A. Talip. All authors reviewed the results and approved the final version of the manuscript.*

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