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Correlation Between Bitterness Removal and Functional Properties of Papaya (*Carica papaya* L.) Leaves

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Abstract— **The mature papaya leaves are known for its medicinal properties albeit its distinctive bitter taste. In this research three different adsorbents; bentonite, activated attapulgite and diatomaceous earth were used to reduce the bitterness of the papaya leaves. The treated papaya leaves were then subjected to sensory evaluation in terms of bitterness intensity and phytochemical compounds which included phenolic, flavonoid, tannin and alkaloid compounds and also antioxidant activity. Papaya leaves treated with bentonite had the most reduced bitterness and application of heat treatment further enhanced the efficacy of bitterness removal. The removal of the bitterness however slightly affected the phytochemical compounds of the papaya leaves and their functional properties. The leaves treated with bentonite by method of boiling was found to be the most effective method in reducing bitterness, with bitterness intensity score of 0.3 out of the original 11.2, while retaining its phytochemical compound; total phenolic content of 10.699 mg GAE/g extract, total flavonoid content of 1.468 mg QE/g extract, total tannin content of 9.423 mg TAE/g extract, total alkaloid content of 1.363% while retained most of its antioxidant IC50 of 1001.058 ppm and antidiabetic properties of 642.231 ppm**

Keywords— **Adsorbent; bitterness removal; Antioxidant activity; Antidiabetic activity**

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I. INTRODUCTION

Papaya, also known as pawpaw, plant belongs to the genus Carica. The edible parts of the papaya plant are the papaya fruit, leaves and flowers. Although the papaya fruit is sweet in taste, the leaves of the papaya plant is known for its bitter taste. Papaya leaves are rich in many phytochemical compounds that contribute to their beneficial function such as: anti-inflammatory properties, wound healing properties, antitumor, immune-modulatory effects and antioxidant [1–4] However papaya leaves also contain some bitter compounds like carpaine and pseudocarpaine, which belong to the alkaloid group, that make the papaya leaves are less desirable for consumption [5]

Bitterness removal in several food products has been a major concern in the food industry. Several methods have been attempted to reduce the bitterness of papaya leaves , e.g. soaking leaves in brine. Another method is by squeezing papaya leaves with salt together with other types of leaves such as guava leaves, followed by boiling. These traditional methods, however, still leave a notable bitter taste. In some part of Sumatra, people used soil to remove the bitterness of the papaya leaves. The leaves are mixed with soil and washed clean prior to heat process to reduce the bitterness and preserve the color of the leaves. The soil contains various clays and minerals that can act as adsorbents. [6].

Various researches have been done to reduce bitterness in different food products such as orange juice and cold pressed grapefruit seed oil by using adsorbents. In cold pressed grapefruit seed oil, the adsorbents were amberlite resins, natural zeolite, sepiolite, and montmorillonite at 3% (w/w) which could reduce the bitterness level of the grapefruit seed oil by half its original bitterness [7], [8]. Another study was done to orange juice by using amberlite resins which could remove limonin, the main component that caused bitterness in orange juice, up to 90% at 30°C and 96% at 40°C. In this research, activated attapulgite, bentonite clay and diatomaceous earth were used as adsorbents to remove or reduce the bitterness of papaya leaves. Heat and simple mechanical treatment like squeezing was applied to increase the efficiency of bitterness removal. Bitterness is often associated with the functional and medicinal properties of natural products. Therefore the attempt of removing bitterness might also reduce the functional and medicinal properties of the papaya leaves. In this research the efficiency of bitterness removal, will be correlated with the change in phytochemical compounds, before and after adsorbent treatment.

II. Material and Methods

A. Material

The materials used in this research were green papaya leaves obtain from local market (approximate diameter of 20 cm), Activated Attapulgite (Diatab - (Mg, Al)□Si□O□□), bentonite (Montmorillonite Merck cas.no 285234) and diatomite (Diatomaceus Earth - Merck cas.no 68855-54-9). For phytochemical analysis, the materials used were ethanol, 10% Folin-Ciocalteu reagent, 7.5% Na_2CO_3, 2% $AlCl_3$, (DPPH) reagent, methanol, 10% acetic acid, 1% ammonium hydroxide solution, α- glukosidase enzyme, p-nitrophenyl- α-D glukopyranoside, Na_2CO_3, phosphate buffer, gallic acid, tannic acid, and quercetin.

B. Methods

Fresh papaya leaves were added with different adsorbent (ratio 1:10) and different application techniques, i.e., boiling, submerging and mechanical squeezing were applied. The treated leaves were divided into two groups. The first one was subjected to sensory panelist and the other was dried using freeze drying followed by extraction using ethanol. The extract was then subjected into various phytochemical analysis , i.e., tannins, flavonoid, total phenolic and alkaloid content. Antioxidant activity of the papaya leaves samples was measured using the DPPH method. While the antidiabetic activity was evaluated by α-glucosidase inhibition method and the IC50 value were calculated for every sample [9], [10] .

Panelist training was conducted to improve the ability of the panelist and their consistency to evaluate the sensory characteristics of the products. Panelists were introduced to the terminology, attributes, and intensity of the bitter taste using caffeinated solution as the reference taste. Elimination screening was conductud after the training to select panelists with the superior ability to discern and score bitterness. There were 50 individuals who were selected the first screening step through personal interview and score in matching test. The number went down to 43 candidates during the second screening step. The second screening test was the triangle tests with increasing difficulties and there were 30 candidates managed to answer at least 60% of the correct answers and went through the last ranking test. Finally there are 23 candidate selected as the trained panelist through their ability to correctly rank the samples in the correct order of bitterness [11–13]

The bitterness test was conducted using a scalar test with a 15 scaled score. Score 0 means the determined taste was not detected, while score 15 means that the determined taste was highly detected [14]. The scalar test was conducted in a standard sensory booth. The panelists were given samples, a questionnaire, and water for rinsing. Each sample was evaluated in duplicate. The collected data were processed using Analysis of Variance (ANOVA) with the IBM SPSS 22.0 software.

III. Result and Discussion

A. Bitterness Intensity

Results of the bitterness intensity perceived by the panelists can be seen on **Table 1.** The ANOVA showed that adsorbent, treatment, and their interaction significantly ($p<0.05$) affected the bitterness intensity of the samples. Most of the leaves treated with adsorbents displayed reduced bitterness intensity compared to the untreated leaves (Table 1). The leaves treated with bentonite clay – boiled showed the most reduced bitterness followed by attapulgite. Bentonite had the biggest specific surface area of 800 m^2/g compared to activated attapulgite and diatomite (125-210 m^2/g and 0.7-180 m^2/g, respectively)[15], [16][17]. This probably explains the stronger adsorption of bitterness by bentonite compared to activated attapulgite and diatomite[6], [17]. Attapulgite was shown to be the least effective in removing bitterness, in which only attapulgite applied with boiling method can reduce the bitterness of the leaves by 86,6 %.

TABLE 1.
BITTERNESS INTENSITY OF SAMPLES

Sample	**Bitterness intensity**
Bentonite boiled	0.3 ± 1.68^{a}
Bentonite squeezed	3.6 ± 0.55^{c}
Bentonite submerged	10.0 ± 0.56^{c}
Attapulgite boiled	1.5 ± 0.59^{b}
Attapulgite squeezed	11.2 ± 0.81^{f}
Attapulgite submerged	12.2 ± 0.82^{g}
Diatomite squeezed	3.3 ± 0.51^{c}
Diatomite squeezed	6.5 ± 0.911^{d}
Diatomite submerged	10.4 ± 0.48^{c}

Note*: Control = 11.2±0.4*
Note: Different notations (letters) indicates a significant difference (p<0.05)

Betonite and diatomite perform better in removing bitterness at room temperature compare to attapulgite. According to Jianhua Huang, 2008[18] attapulgite can only reduce bitterness if applied in the presence of heat as the adsorption process occurs spontaneously in high temperature. Hence as shown in the result, attapulgite is not effective in removing bitter taste at room temperature. All the treated samples were then subjected to phytochemical analysis and the results were compared to the fresh samples.

B. Phytochemical Analysis of Treated Papaya Leaves.

Adsorbent, treatment, and their interactions gave significant affects ($p<0.05$) to total alkaloid and total phenolic contents (Table 2). From the result, it can be seen that the application of adsorbent with additional heat treatment reduced the most amounts of phytochemical compounds compared to other treatments. Heat can assist the adsorption of organic compounds, thus helping the phytochemical removal of the leaves. This trend can be seen in all types of adsorbents used in the research.

As for the type of adsorbent; bentonite and diatomite are more superior in reducing both alkaloid and phenolic compounds compared to attapulgite. The reduction of alkaloid content of the treated samples was related to the specific surface area of the adsorbents. Greater specific surface area enables the adsorbent to adsorb more compounds. Among the three different adsorbents, bentonite has the highest specific surface area (800 m^2/g), followed by activated attapulgite (125-210 m^2/g) and lastly diatomite (0.7-180 m^2/g) [17], [19] Moreover, diatomaceous earth contains 85.5-91.8% of silicon dioxide while the amount of SiO_2 in bentonite clay ranges from 56-74%. Silica contains silanol functional groups on its surface. Phenol groups are able to bind with silica through hydrogen bonding between the silanol group and OH group of phenolic compounds [17]. Therefore increase the adsorption of the phenolic compound into the diatomite.

Similarly, the application of adsorbents also reduced the flavonoid contents (**Table 2**). Similar with the total alkaloid and phenolic content, Type of adsorbent and application treatment gave significant affects ($p<0.05$) towards the total flavonoid content and total tannin content. Bentonite and diatomite easily bind flavonoid thus resulting in the decrease of flavonoid content in the treated papaya leaves. Similar trend can also be observed in tannin content, in which the application of heat also increased the amount of tannin adsorbed in the adsorbent. Tannin has the tendency to bind proteins [20] similarly with bentonite and diatomite. Meanwhile attapulgite has the ability to adsorb tannins through hydrogen bonding [18]. Therefore, tannins may be removed during the adsorption

TABLE 2
PHYTOCHEMICAL ANALYSIS OF PAPAYA LEAVES AFTER ADSORPTION TREATMENT

Sample	Bitterness intensity	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QUE/g)	Total Tanin Content (mg TAE/g)	Total Alkaloid (%)
B. boiled	1.363±0.622[a]	10.699±1.656[a]	1.468±0.112[a]	898.984±2.4[b]	1.363±0.622[a]
B. squeezed	1.835±0.292[a]	17.396±0.715[b]	4.749±0.670[d]	970.786±6.0[c]	1.835±0.292[a]
B. submerged	9.020±0.831[c]	28.462±4.362[c]	3.769±0.550[c]	1001.058±9.0[e]	9.020±0.831[c]
A. boiled	1.782±0.292[a]	10.460±1.602[a]	3.872±0.494[cd]	827.458±2.2[a]	1.782±0.292[a]
A. squeezed	12.381±0.531[d]	11.368±0.499[a]	2.545±0.704[b]	980.411±2.9[d]	12.381±0.531[d]
A. submerged	19.466±2.751[f]	36.008±1.793[d]	8.560±1.731[e]	1011.700±2.3[f]	19.466±2.751[f]
D. Boiled	2.641±0.566[a]	9.515±2.174[a]	3.314±0.844[bc]	809.373±7.1[b]	2.641±0.566[a]
D. squeezed	5.266±1.404[b]	9.944±1.991[a]	3.349±0.245[bc]	1012.270±3.2[f]	5.266±1.404[b]
D. submerged	14.227±2.168[e]	17.528±1.935[b]	3.990±0.355[cd]	1014.455±9.9[f]	14.227±2.168[e]
Control	11.2±0.423	30.716±0.881	10.260±0.670	832.940±4.3	11.2±0.402

[*)] Control are untreated leaves, B (Bentonite), A (Attapulgite), D (Diatomite)
Note: Different notations (letters) indicates a significant difference (p<0.05

C. Correlation between bitterness intensity and functional properties of papaya leaves

The adsorbent, treatment, and their interactions affected the antioxidant properties and the antidiabetic properties of the sample ($p<0.05$) as seen in Table 4. Higher IC_{50} values indicate lower activities for both antioxidant and antidiabetic properties [9], [10].

TABLE 3.
RADICAL SCAVENGING AND ANTIDIABETIC ACTIVITIES (IC_{50}) OF TREATED PAPAYA LEAF SAMPLES

Sample	Radical Scavenging Activity	Antidiabetic Activity
B. boiled	1001.058±9.0[e]	642.231 ± 6.7[ef]
B. squeezed	970.786±6.0[c]	596.245 ± 11.1[cd]
B. submerged	898.984±2.4[b]	629.72 ± 11.9[def]
A. boiled	1011.700±2.3[f]	703.07 ±16.3[g]
A. squeezed	980.411±2.9[d]	638.974 ± 3.8[ef]
A. submerged	827.458±2.2[a]	663.514 ± 12.0[f]
D. Boiled	1014.455±10[f]	607.999 ± 26.3[cde]
D. squeezed	1012.270±3.2[f]	511.542 ± 80.6[b]
D. submerged	899.373±7.1[b]	588.148 ± 9.9c
Control	832.940±4.3[a]	257.134 ± 10.4a

Note: Different notations (letters) indicates a significant difference (p<0.05)

Heat-treated sample resulted in the lowest antioxidant activity compared to the other two methods. Heat treatment allows molecules to move faster thus resulting in better adsorption process. Therefore, the major removal of phytochemical compound was observed in the heat treated sample [9], [21] process regardless the methods of application and type of adsorbents. The application of heat might increase the adsorbing reaction to some degree by increasing the value of BET-Surface area of a silica based adsorbent. In the research published by Waseem et al 2017, the BET Surface area of several silica based adsorbent are increase during low heat treatment (100-200 ^{0}C) which might explain the decline in the antioxidant activity[22].

Adsorbent also affected the antioxidant activity of the treated samples ($p<0.05$). The leaves that loss many of its phytochemical properties also loss some its antioxidant properties or at least it's radical scavenging activities. Overall, attapulgite and bentonite treated samples retained the antioxidants activities; meanwhile leaves that were treated using diatomite has the lower radical scavenging value.

For the antidiabetic properties, it can be seen that all leaves that undergone adsorbent treatment have lower antidiabetic properties. Treated sample that have greater reduction in phytochemical content also displayed significant increase in the IC_{50} of the treated sample compare to the control which indicate the decrease in antidiabetic properties. The antidiabetic method that is used in this research rely on the ability of the extract to inhibit the activity of α-glucosidase, an enzyme that presence in the cell lining of the cell that control the adsorption of sugar. However there are several studies that showed papaya leaves are also helpful in decreasing secondary complications of diabetes by improving insulin sensitivity to uptake glucose by cells [10], [23] which is not be reflected by the antidiabetic methods used in this research. The papaya leaves might still have antidiabetic activity that was not represented in this research.

Bitterness is believed as the sensorial perception from several phytochemical, thus the removal of the phytochemical lead to reduced bitterness intensity from the papaya leaves. This study showed that alkaloids were positive (0.935) and highly correlated with bitterness intensity (Table 4) which suggested that these compounds were reasons behind the bitterness of papaya leaves[5]. The removal of the phytochemical also correlated to the functional properties of the papaya leaves, as seen in the table below. Both Antioxidant activity and Antidiabetic have positive correlation with the bitter taste of the papaya leaves.

Antioxidant activities of papaya leaves were mainly contributed by the phenolic, flavonoid and alkaloid contents, while antidiabetic activities were mainly due to flavonoids content [2][3]. Therefore the reduction of the phytochemical will also affected both functional properties of the papaya leaves. However a closer look to the IC_{50} values of antioxidant and antidiabetic in table 3, showed that the loss of both antioxidant activity and antidiabetic activity were effectively lower than the loss in bitter taste, showing the effectiveness of the treatment.

TABLE 4.
CORRELATION BETWEEN ANTIOXIDANT ACTIVITY, BITTERNESS AND PHYTOCHEMICAL COMPOUNDS

	Phenolic	**Flavonoid**	**Tannin**	**Alkaloid**	**Bitterness intensity**
Antioxidant activity	.951**	.827**	.648*	.868**	.779**
Antidiabetic Activity	.617	.888**	.583	.534	.671*
Bitterness Intensity	.552	.686*	.404	. 935**	1

Note: * correlation is significant at the p<0.05 level
** correlation is significant at the p<0.01 level

IV. CONCLUSION

Papaya leaves that were cooked using different combinations of adsorbents and treatments had lower amounts of phytochemical compounds and weaker bitterness intensity compared to untreated leaves. The most reduced bitterness intensity was achieved by leaves treated with bentonite. In terms of the methods of application, boiling resulted in the weakest bitterness intensity compared to submerged and squeezing methods.

The loss of phytochemical compound such as phenolic, flavonoids, tannins, alkaloids cause the reduction in bitterness intensity and both antioxidant activity of papaya leaves and antidiabetic properties. The application of Adsorbents had the ability to remove most of the phytochemicals that were associated with bitterness in papaya leaves which showed the potential use of adsorbents in removing the bitterness contained in papaya leaves. Although the antioxidant and antidiabetic properties were compromised, the better palatability of treated papaya leaves will lead to increased daily intake, thus increase the potential of papaya leaves as medicinal natural products.

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