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Optimization of Aqueous Extraction of Indonesian Bay Leaf (Syzygium polyanthum Wight) as Powder Seasoning

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ABSTRACT

Indonesian bay leaf (*Syzygium polyanthum* Wight) is one of traditional spices originated from Indonesia which is usually used as spices to add the aroma of foods. Extraction of the aroma of Indonesian bay leaf could be proposed as a more practical usage to its fresh form. This research aims to find the optimum condition of the extraction of the aroma compounds from *S. polyanthum* and further dry the extract using a spray dryer. The *S. polyanthum* were extracted using water in extraction method (using ultrasound bath, water bath shaker, hotplate and stirrer, reflux condenser) followed by variation in extraction time (2, 4, and 6 hours) and temperature (50, 60, 70, and 80 °C). The extracts were evaluated for their total soluble solid, sensory acceptance, and carbonyl compound content with DNS assay which pointed out a combination of heating and stirring at 50 or 80 °C for 2 hours as optimum extraction condition. Further evaluation using GC-MS, sensory evaluation technique, DNS assay, moisture content, color, and solubility time of the spray dried powder from bay leaves extracted at 50 (P50) and 80 °C (P80) showed that both powder have significant difference in both sensory and physical characters as well as aroma compound composition. P80 which contain oxalic acid, icosane, 2-hexylthiophene, and n-hexadecanoic acid, were found to have higher overall sensory acceptance.

Keywords: *aroma, aqueous extraction, bay leaf, spray dry, Syzygium polyanthum Wight*.

1. INTRODUCTION

The global demand for spices has increased along with the development of the processed food industry [1] Powder forms of spices are used in exports because they are easy to be transported and unsusceptible to microbial contamination due to its low water content and activity.

Indonesian bay leaf (*Syzygium polyanthum* Wight), that belongs to the family *Myrtaceae,* is a common spices used in Indonesian cuisine to provide a unique aroma and slightly astringent taste to the food, though it can also be used as a remedy for diarrhea, diabetes and also high blood pressure [2]. While it is commonly used in its fresh form in Indonesian cuisine, *S. polyanthum* is also distributed as spices in its dry form outside Indonesia. However, drying, though successfully extending the shelf life, causes many losses in the sensory characters [3]. The preparation of ready-to-use flavouring ingredients that retain its original flavor profile remains a challenge in the food industry.

Several studies have been done in the extraction of aromatic compounds from *S. polyanthum*. Extraction of *S. polyanthum* using water, ethanol, and mixture of water and ethanol yields a comparable amount of phenolic compounds that are responsible for the aroma and astringent taste [4]. Steam distillation was also shown to yield higher aromatic compounds compared to n-hexane extraction [5]. The studies pointed out the promising use of water for extraction media. Moreover water is a common medium in traditional cooking, easily available, non-toxic, and easily recycled or disposed of with minimum environmental problems [6]. Nevertheless, it is quite important to further optimize the extraction condition and to preserve the extract. While freezing is a common method to preserve extract, it is not very convenient for distribution. Spray drying is a common method in the liquid extract preservation and it is specially efficient upon water extract.

This research aims to find the optimum condition which resulted in a high concentration of aroma compounds of Indonesian bay leaf followed by a spray

drying process to preserve the aroma compound while extending its shelf-life.

2. MATERIALS AND METHOD

Raw materials utilized in this research were Indonesian Bay Leaf which were harvested from Tangerang City, Indonesia. The chemicals used for extraction and analysis were distilled water (PT. Amidis Tirta Mulia, Indonesia), sodium hydroxide (Merck, Germany), potassium sodium tartrate tetrahydrate (Merck, Germany), 3,5- dinitrosalicylic acid (DNS, Biobasic Inc, Canada), D(+)-glucose (Merck, Germany), maltodextrin (Asian Chemical, Indonesia).

The research designs were separated into three stages: (1) extraction method determination, (2) optimization of extraction condition, and (3) pulverization. In the first stage, the bay leaves were extracted at 70 °C for 2 hours using ultrasonic bath (Bandelin Electronic, Germany), water bath shaker (Memmert, Germany), hotplate with magnetic stirrer, and reflux condenser (Haake, Germany). The resulting extracts were then evaluated for their total soluble using a refractometer (Atago, Japan) and evaluated for its aroma intensity by sensory evaluation with a ranking method. The ranking test employed 30 untrained panelists that were requested to rank "1" for extract with strongest aroma and rank the rest accordingly.

In the second stage, bay leaves were extracted using the chosen method in various time (2, 4, and 6 hours) and temperature (50, 60, 70, and 80 °C). Samples were evaluated using DNS assay for reducing sugar (Miller, 1959) to evaluate the amount of compounds with carbonyl compounds that represent the aroma compound of *S. polyanthum*. Per liter DNS reagents mixture contain 10 g of sodium hydroxide, 182 g of potassium sodium tartaric acid, and 10 g of DNS. The DNS reagent mixture and the *S. polyanthum* extract (1:1) were allowed to react in boiling water for 5 min and then evaluated using uv-vis spectrophotometer (Genesys 10uv Thermo Electron Corporation, USA) at 540 nm. The

The DNS reagents were made by diluting 5 grams of Sodium Hydroxide in 250 mL distilled water and 91 grams of Potassium Sodium Tartaric Acid was added into the mixture. 5 grams of DNS were then added into the mixture and the volume of the mixture was made until 500 mL. The DNS reagent would then be added to the sample with 1:1 ratio. The sample and DNS mixture were then allowed to be incubated in boiling water for 5 minutes. The mixture was then cooled down and then analyzed further using the UV-Vis Spectrophotometer using the wavelength of 540 nm. The absorbance was then evaluated using the glucose standard curve to yield the concentration of carbonyl compounds in the glucose equivalent (GE)/ml sample.

Selected extraction methods and conditions (time and temperature) were then applied to extract a larger amount of *S. polyanthum*. In the third stage, the resulting extract was then added with 50 g maltodextrin per liter of extract and then evaporated at 70 °C until the total soluble solid reached 20%, using vacuum rotary evaporator (IKA, China). The slightly concentrated extracts were then injected to the spray dryer (Buchi, Germany) with inlet and outlet temperatures of 180 and 80 °C, respectively. The resulting spray dried powder was compared for its carbonyl compounds using DNS assay as well as its overall sensory difference and acceptance in comparison to the prior-drying-extracts. The aromatic compounds from the spray-dried powders were also evaluated using GC-MS (Agilent Technologies, USA). The powder characters were evaluated using solubility time analysis, colorimeter (PCE Instruments, Germany), and moisture content analyzer (Sartorius, Germany)

3. RESULTS AND DISCUSSION

Several extraction methods were observed for its efficiency in extracting soluble solid as well as the aroma compound from *S. polyanthum*. The bay leaves extract would be further processed into powder by spray drying and total soluble solid is one of the critical parameters for spray drying [7]. Comparison between aqueous extract resulted from extraction at 70 °C for 2 hours using ultrasonic bath, water bath shaker, hotplate and magnetic stirrer, and reflux condenser pointed out heating and stirring (MS) as the most effective method as it resulted in the highest TSS of 1.47 % (Table 1). The aroma intensity ranking test also showed that MS has the highest intensity as shown with lowest score of rank number of 2.07 (Table 1). Therefore water extraction using hotplate and magnetic stirrer was chosen for the extraction method in the next stage of the research.

Several bay leaves aqueous extract with extraction using hotplate and stirrer at several temperatures (50, 60, 70, and 80 °C) for several periods of time (2, 4, and 6 hours) were compared using DNS assay. DNS assay tests the presence of carbonyl compounds, such as the major flavor compound found in bay leaves, namely cis-4-decenal, octanal, nonanal [5]. Higher and longer heat exposure may cause compound damage through pyrolysis and hydrolysis [8], explaining that the extract MS-50-2 with lowest extraction time and temperature (2 hours, 50 °C) yields higher carbonyl compound estimated content (Figure 1). Nevertheless the extract MS-80-2 that applied a much higher temperature (80 °C) were able to yield comparable carbonyl compound estimated content (Figure 1).

Table 1 Total soluble solid (TSS) and mean score of aroma intensity ranking test of the bay leaves aqueous extract with several extraction method

Extraction at 70 °C, 2h using	Extract code	TSS (% Brix)	Aroma intensity rank score*
ultrasound sonicator	US	1.27 ± 0.06 [a]	2.13 ± 0.90 [a,b]
water bath shaker	WS	1.13 ± 0.06 [a,b]	3.47 ± 1.01 [c]
hotplate and magnetic stirrer	MS	1.47 ± 0.06 [b]	2.07 ± 0.87 [a]
reflux condenser	RC	1.33 ± 0.06 [b]	2.33 ± 1.12 [b,c]

Results are shown in mean ± standard deviation. Different letter in the same **column** indicate significant differences at $p > 0.05$
*Lower number indicate higher rank of aroma intensity

High temperature extraction may cause compound alteration [9], that may form new compounds that also contain carbonyl groups. In agreement, the triangle test also indicated that MS-50-2 and MS-80-2 overall sensory characters are significantly perceivable (at $p > 0.05$), as shown with higher actual correct responses compared to the minimum required of correct response (Table 2). Both MS-50-2 and MS-80-2 were used in the next spray drying stage to produce powders from the bay leaves aqueous extract.

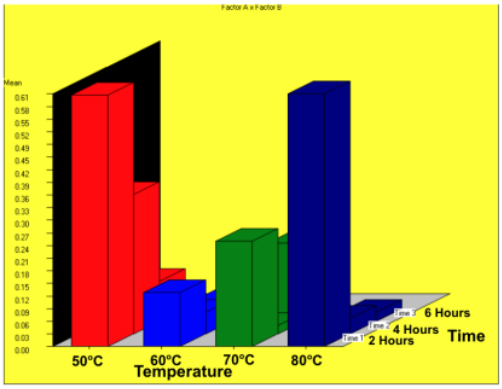


Figure 1 Estimated carbonyl compound content (mg GE/ml) of the bay leaves aqueous extract with extraction using hotplate and stirrer at several temperatures (50, 60, 70, and 80 °C) for several periods of time (2, 4, and 6 hours)

The chemical evaluation of of carbonyl compound estimation using DNS assay and major volatile compounds composition using GC-MS assay showed that powder from bay leaves aqueous extracts with extraction using hotplate and stirrer for 2 hours at 50 (MS-50-2-P) and 80 °C (MS-80-2-P) further confirmed the flavor compounds differences (Table 3). MS-50-2-P were shown to have higher estimated content of carbonyl compounds (1.90 mg GE/ml) but MS-80-2-P were found to have more variation of volatile compounds elucidated. Both powders were shown to have oxalic acid, but the rest volatile compositions were different (Table 3).

Table 2 Responses in the overall sensory difference and acceptance tests of bay leaves aqueous extracts with extraction using hotplate and stirrer for 2 hours at 50 (MS-50-2) and 80 °C (MS-80-2)

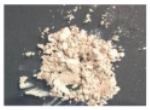
Type of Evaluation	Bay leaves extracts	Type of response	Number of response
Overall sensory difference (Method: Triangle Test)	MS-50-2 MS-80-2	Correct	21
		Incorrect	9
		Total Response	30
		Minimum correct response to be significant at p 0.05 (Meilgaard et al., 2017)	15 out of 30
Type of Evaluation		**Bay leaves extracts**	**Mean Score***
Overall sensory preference (Method: Hedonic Test)		MS-50-2	5.86 ± 1.19[a]
		MS-80-2	6.17 ± 1.96[a]

* Results are shown in mean ± standard deviation (1= extremely dislike, 9= extremely like. Different letter in the same **column** indicate significant differences at $p > 0.05$

As shown in Table 3, both powders were also different in terms of physical characteristics. MS-80-2-P was slightly darker (< L*) but brighter (< *C) color, with 2 degree of hue difference (Δh) toward more red (> a*) and more yellow (> b*) color. Total difference (ΔE) between the two samples was 3.10, which are defined to be perceptible at a glance. MS-50-2-P have higher moisture content (6.1 %), require less time to be diluted (49%) and have higher hygroscopicity as compared to MS-80-2-P. The MS-50-2-P powder is very easy to be diluted and it clumps easily upon exposure to open air. Nevertheless, just like the original extracts, no significant differences were recorded for the overall sensory preference between MS-50-2-P and MS-80-2-P (Table 3)

Table 3 Chemical, sensory, and physical characters of spray-dried powders from bay leaves aqueous extracts with extraction using hotplate and stirrer for 2 hours at 50 (MS-50-2-P) and 80 °C (MS-80-2-P)

Observed Character	MS-50-2-P	MS-80-2-P
Carbonyl compound (mg GE/ml)	1.90 ± 0.01[a]	1.24 ± 0.04[b]
Major volatile compounds (GC-MS)	Oxalic acid 2-Butyl thiophene Sulfurous acid cyclohexylmethyl hexadecyl ester	Oxalic acid 2-Thiopheneacetic acid, 2-tridecyl ester

	Cyclohexane propionic acid	Thiophene, 2-hexyl 3-decyn-1-ol Icosane Palmitic acid
Visual appearance		
Color index		
Lightness (L*)	70.929[a]	69.369[b]
Red/green (a*)	5.580[a]	7.249[b]
Yellow/blue (b*)	10.284[a]	12.380[b]
Chroma (C*)	11.703[a]	14.347[b]
hue (h*)	61.606[a]	59.655[b]
Color difference (ΔE)	3.10	
Moisture content (%)	6.1 ± 0.2[a]	3.4 ± 0.3[b]
Solubility time (s)	49 ± 3[a]	64 ± 4[b]
Hygroscopicity	+++	+
Hedonic score*	5.58 ± 1.32[a]	5.94 ± 1.17[a]

Different letter in the same **row** indicate significant differences at $p > 0.05$

* Results are shown in mean ± standard deviation (1= extremely dislike, 9= extremely like.

4. CONCLUSION

Heating and stirring combination is a highly effective method in the aqueous extraction of bay leaves. The optimum condition for bay leaf extraction in this study was the lowest time and temperature variation (2 hours, 50 °C), though the highest temperature variation also showed high extraction efficiency. Chemical evaluation showed differences in the flavor compound composition of both extract. Both extracts also have perceptible differences in their powder form. Nevertheless, both extract acceptance rates were not significantly different.

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