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Characteristics of Effervescent Granules Extract of Kenikir (*Cosmos caudatus* Kunth) Leaf with Various Acid Compositions as Alternative Functional Beverage Products

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A B S T R A C T

Kenikir (*Cosmos caudatus* Kunth) leaves are plants that contain active compounds that function as antioxidants and have the potential as functional drinks. This research was conducted with the aim of knowing the effect of using a combination of acid sources between citric acid and tartaric acid on the characteristics of effervescent granules, to obtain the best combination of acid sources. The stages of this research were the effervescent granule formulation of kenikir leaf extract including the extraction process, microencapsulation, and determination of the formula with a combination of citric acid and tartaric. The method used for the making of effervescent granules was dry granulation method. The factor used in this research was the ratio of the composition of citric acid and tartaric acid including 0%:100%, 25%:75%, 50%:50%, 75%:25% and 100%:0%. The experimental design used in this study was a randomized block design. The data were analyzed by means of variance and continued with the Tukey test if there was an effect of treatment on the results. The results showed that the comparison of the composition of citric acid and tartaric acid had a significant effect on the characteristics of effervescent granules of kenikir leaf extract. The best composition of citric acid and tartaric acid is 75% citric acid and 25% tartaric acid which produces specific gravity 41g/ml, bulk density 0.72gr/ml, flow rate 12, 39gr/second, dissolution time 137seconds, foam volume 8%, pH 6.32 and has antioxidant activity of 21.37%.

Introduction

One of the plants that has the potential as a source of functional drinks is kenikir (*Cosmos caudatus* Kunth). Kenikir plants are generally only used as fresh vegetables or as appetizers. The part of the plant that is commonly used is the leaves. Kenikir (*Cosmos caudatus* K.) is a plant that can be found in Central America and Southeast Asia, and is one of the vegetables that is often consumed as fresh vegetables by eating or chewing directly (Cheng *et al.*, 2015). The content of the ethanol extract of kenikir leaves in the research of Dwiyanti *et al.*, (2014) showed the presence of active compounds in the form of flavonoids, saponins, terpenoids, alkaloids, tannins, essential oils and vitamin C. Ethanol extract from kenikir leaves has antioxidant activity with an IC_{50} value of 72µg/ml and a phenolic compound content of 377.1 mg GAE/g (Rahman *et al.*, 2016).The use of kenikir leaves in the wider community, which are generally eaten directly as whole leaves, is lacking of selling value and good innovation to maximize the nutrition contained. The right formulation in processing natural ingredients into a form that is easily accepted by the public is expected to improve product quality as a diversification of functional food and beverage presentation forms, increase practicality and public interest in consuming functional products. One effort to improve this is to make kenikir leaves into effervescent granules. Effervescent is a product that contains a mixture of acidic and basic carbonate substances that can react quickly when it meets water and produce foam or bubbles of carbon dioxide (CO_2) gas and causing a refreshing sparkle effect. Effervescents are generally processed using a combination of acid sources because the use of a single acid source will complicate the process of making effervescents (Lieberman, *et al.*, 1994). An important consideration in the processing of effervescent granules is the determination of the appropriate acid-base components.

In this research, the processing of effervescent granules was carried out using two sources of acid, namely citric acid and tartaric acid. This research was conducted with the aim of knowing the effect of using a combination of acid sources between citric acid and tartaric acid on the characteristics of effervescent granules, and to obtain the best combination of acid sources.

Materials and Methods

This research was carried out at the Engineering Laboratory, Food Analysis Laboratory, Faculty of Agricultural Technology, Udayana University and the Center for Marine Cultivation Research and Fisheries Extension, Gondol, Singaraja. During the period of December 2020 to April 2021.

The main ingredients used are young and fresh kenikir (*Cosmos caudatus* K.) leaves with green leaf and taken from one to four levels below the shoot. The chemicals used were distilled water (Rofa, Indonesia), DPPH pro analysys (Sigma-Aldrich, USA) ethanol 96%, NaNO2 5% (Merck, Germany), 10% AlCl3 (Phyfo Technology Laboratories, USA), NaOH 4% (Merck, Germany), gallicacid (Sigma-Aldrich, USA), quercetin (Sigma-Aldrich, USA), NaNO2 (10%), Follin Denis reagent (Merck, Germany), maltodextrin, Na. Casinate, citric acid, tartaric acid, Na. Bicarbonate, lactose, sucrose, aquadest, PEG 6000.

Material preparation

Kenikir leaves are sorted and washed first with running water, then reduced in size to be crushed using a blender, then dried in a cabinet dryer at a temperature of 50°C to a 5% of moisture content. After that, the dried leaves are mashed with a dry blender and sieved with a 40 mesh sieve. The obtained powder is ready to be used for research.

Extraction

Kenikir leaf powder was extracted using maceration method with 96% ethanol (1:10) for 24 hours and filtered with Whatman No.1

paper. The filtrate was concentrated by means of a rotary vacuum evaporator with a temperature of 55°C, a rotational speed of 65 rpm and a pressure of 100mbar, the method refers to the research of Putranto *et al.,*(2018) which has been modified.

Microencapsulation

As much as 30 grams of maltodextrin and Na caseinate were prepared in a ratio of 80:20, then put into a volumetric flask and added with distilled water to a volume of 300ml, to form an encapsulated solution. The extract was added 1% of the encapsulated solution and continued with the homogenization process at a speed of 11000 rpm for 60 minutes. The mixture was dried in a freeze dryer at -75^{o}C for 3 days.

Effervescent granule formulation

The effervescent granule formulation of *kenikir* leaf extract in this study used the ratio of the composition of the acid source (citric acid: tartaric acid) namely 0%:100%, 25%:75%, 50%:50%, 75%:25% and 100%:0 %. Details of the formulation are shown in Table 1.

Analysis

The resulting effervescent granules were then physically analyzed or characteristic tests included specific gravity, bulk density, flow rate, dissolution time, foam volume and pH. The formula used refers to the research of Chabib, *et al.,* (2015) which has been modified.

Research Design

This research used a Randomized Block Design (RAK) with one factor, namely the comparison of the composition of the acid source, between citric acid and tartaric acid with 5 levels of treatment. Each treatment was carried out three times based on the processing time and 15 experimental units were obtained. Data analysis was carried out using the Minitab 19 software.

Data Analysis

The data obtained were analyzed using ANOVA (*Analysis of Variance*) (Sugiyono, 2011). If there is a significant effect (level 5%) then the analysis is continued by testing the mean difference between treatments with the Tukey multiple comparison test.

Results and Discussion

Specific gravity

The results showed that the composition of citric acid and tartaric acid had a very significant effect ($p<0.01$) on the specific gravity of the granules (Table 2). Specific gravity resulting from the addition of 0% citric acid composition and 100% tartaric acid ie 0.41 g/ml was significantly different from the composition of 100% citric acid and 0% tartaric acid which was 0.50 g/ml. A good specific gravity value is 0.2 - 0.6 g/mL (Goeswin, 2012).

In this result, all formulas had specific gravity in accordance with research conducted by Goeswin (2012). Specific gravity is one of the parameters that play a role in determining the level of solubility or solubility of a substance (Juniarti, 2009). Weight is one of the parameters that play a role in determining the level of solubility or solubility of a substance (Juniarti, 2009).

Bulk density

The results showed that the composition of citric acid and tartaric acid had a very significant effect ($p<0.01$) on the granules bulk density (Table 2). The bulk density produced in the composition of 0% citric acid and 100% tartaric acid, which is 0.66 g/ml, is significantly different from the composition of 75% citric acid and 25% tartaric acid, which is 0.72g/ml. The bulk density values in all formulas are close to the best bulk density numbers, good bulk density values are close to 1 (Jufri, 2006). In the results of this study, granules with more citric acid composition than tartaric acid have a higher bulk density.

The bulk density is very important in determining the size of the container required during the handling, shipping and storage of raw materials and mixtures. The bulk density is also important in the size of the mixing equipment (Hamsinah *et al.,* 2020). The bulk density of all formulas showed low values and it can be assumed that the resulting effervescent granules required smaller containers.

Flow rate

The results showed that the composition of citric acid and tartaric acid had a very significant effect ($p<0.01$) on the flow rate of the granules (Table 2). The use of a single acid resulted in a slower granule flow rate compared to the use of the composition of two acid sources, namely citric and tartaric acid. The addition of more citric acid composition than tartaric acid resulted in a better flow rate.

In the formulation without citric acid and without tartaric acid, the flow rate decreased drastically. The flow rate resulting from the addition of the composition of citric acid and tartaric acid in each formula was significantly different with an average granule flow rate of 10.35g/second to 12.39g/second. The flow rate is the time required for the granules to flow freely after being poured into a device. Granule flow rate indicates the number of granules flowing every second. The factors that affect the granule flow time are particle size, particle shape and humidity. Larger, rounder particles indicate better flow.

The flow rate of granules is influenced by the shape of the granules and the size of the granules (Kusuma *et al.,* 2014). The larger the granule size, the better the flow rate, a good flow rate is also influenced by the cohesiveness of the granules. The granules produced in this study were stored at 4-5°C and 40% RH, so as to minimize friction and attraction between the granules. Granule flow is good if the time required to flow 100 grams 10 seconds or 10 grams/second (Anshori *et al.,* 2009). The more granules that can flow in each second the better.

Dissolution time

The results showed that the composition of citric acid and tartaric acid had a very significant effect ($p<0.01$) on the dissolving time of the granules (Table 2). The dissolution time resulting from the composition of 0% citric acid and 100% tartaric acid was 144 seconds, not significantly different from the composition of 100% citric acid and 0% tartaric acid, which was 146 seconds, but significantly different from the composition of 75% citric acid and 25% tartaric acid which is 137 seconds. Dissolution time can be affected by several factors, such as specific gravity, temperature in water, pressure force when dissolving granules.

The dissolution time of a good effervescent powder is not less than 2 minutes and or not more than 4 minutes (Mohrle, 1989). The higher the amount of citric acid will decrease the hardness, increase the friability and decrease the dissolution time of effervescent tablets of kencur extract (Juniawan, 2004).

Table.1 Kenikir leaf extract effervescent granule formulation

Ingredients (mg)	F1	F2	F3	F4	F5
Extract microcapsule	150	150	150	150	150
Citric acid	0	100	200	300	400
Tartaric acid	400	300	200	100	0
Lactose	2840	2840	2840	2840	2840
Sodium bicarboncate	400	400	400	400	400
PEG6000	60	60	60	60	60
Sucrose	150	150	150	150	150
Total	4000	4000	4000	4000	4000

Table.2 Physical evaluation of kenikir leaf extract effervescent granules

Granules physical properties	Formula F1 (0:100)	Formula F2 (25:75)	Formula F3 (50:50)	Formula F4 (75:25)	Formula F5 (100:0)	Requirement/ Literature
Specific gravity (g/ml)	0,41± 0,01b	0,41± 0,01b	0,45± 0,02b	0,41± 0,01b	0,50± 0,01a	0,2 - 0,6 g/mL (Goeswin, 2012)
Bulk density (g/ml)	0,66 ± 0,01ab	0,64 ± 0,01b	0,66± 0,02b	0,72± 0,02a	0,68 ± 0,02ab	approach 1. (Jufri,2006)
Flow speed (seconds)	10,35 ± 0,01e	11,10 ± 0,01c	11,37± 0,01b	12,39 ± 0,01a	10,53 ± 0,02d	100 g granul< 10 seconds (FudholicitSantoso, 2006)
Dissolution time (detik)	144 ± 1,73c	158 ± 1,53b	165± 1,53a	137 ±1,15d	146 ± 1,00c	2-4 minutes (Mohrle, 1989)
Foam volume (%)	12,33 ± 0,29a	13,00 ± 0,50a	11,33± 0,58b	8,00 ± 0,50c	7,17 ± 0,29c	-
pH	6,48 ± 0,02a	6,41 ± 0,01b	6,38± 0,02b	6,32 ± 0,03c	6,21 ± 0,02d	-

Remark : Different letters behind the values indicate significant differences in the BNJ test with a 95% interval. The formula used is citric acid: tartaric acid.

The dissolution time with various acid sources showed that the treatment using two types of acid (citric: tartaric) had the lowest dissolution time among the treatments using one or three types of acid. Citric acid provides a shorter soluble time than tartaric acid, which can be caused by the hygroscopic nature of citric acid which can be more soluble in water (Widyaningrum *et al.*, 2015).

Foam Volume

The results showed that the composition of citric acid and tartaric acid had a very significant effect ($p<0.01$) on the foam volume of the granules (Table 2). The foam volume produced from the composition of citric acid and tartaric acid respectively 0%:100% and 25%:75% was not significantly different, namely 12.33% and 13%. However, the composition of 0% citric acid and 100% tartaric acid was significantly different from the composition of 100% citric acid and 0% tartaric acid, namely 12.33% and 67.17%, respectively.

The use of single tartaric acid forms more carbon dioxide than citric acid because it has a slower soluble time. Citric acid with a higher composition than tartaric acid produces less foam because it has a shorter soluble time. The time required to reach saturation conditions varies from this research, when a treatment reaches saturation conditions quickly, the bubbles will stop producing foam, so that the resulting foam will be less. On the other hand, if the time required to reach saturation is slow, the bubbles will continue to accumulate into foam, so that more and more foam will be produced. This indicates that the foam produced is proportional to the dissolving time, where if the dissolution time is faster, the foam produced is also less.

pH

The results showed that the composition of citric acid and tartaric acid had a very significant effect ($p<0.01$) on the pH of the granules (Table 2). The degree of acidity produced in the composition of citric acid and tartaric acid of 25%:75% and 50%:50%, respectively, was not significantly different, namely 6.41 and 6.38. However, the compositions of 0% citric acid and 100% tartaric acid were significantly different from the compositions of 100% citric acid and 0% tartaric acid, namely 6.48 and 6.21.

The pH possessed in each formula is close to neutral pH, this can be caused by the use of various alkaline compositions, such as sodium bicarbonate and lactose which are not acidic (pH 6.6). The increase in pH is influenced by sodium bicarbonate during formulation which when reacts with an acid source and water becomes decomposed and releases free Na^+ ions. These ions bind to organic acids and form sodium bicarbonate salts so that the activity of H^+ ions in organic acids is lost. The loss of H^+ ion activity in solution causes the OH^- ion activity to be more dominant so that the product is alkaline (Fardiaz, 1989). According to the 2010 Minister of Health Regulation, the recommended pH that has a smallest difference from the standard pH of beverage products is pH 7.

Determination of the formula with the best acid source composition

The formulation with the best acid composition was determined based on the flow rate and dissolving time. The advantages of effervescent products include providing convenience in consuming by providing a pleasant taste. Ease of consumption is very dependent on the speed in the process of dissolving the granules in water.

The shorter the time required, the better, therefore flow rate and dissolution time are important characteristics in effervescent granule formulations because granules with short flow rates will have fast dissolution times. Formula F4 with a composition of 75% citric acid and 25% tartaric acid produced the shortest flow rate and dissolving time compared to other compositions, namely 12.39g/second and 137 seconds (2 minutes 28 seconds) and has antioxidant activity of 21.37%.

In conclusion, the use of the acid source composition had a very affected for all the physical characteristics of the effervescent granules of *kenikir* leaf extract. They are specific gravity, bulk density, flow rate, dissolving time, foam volume and pH. The composition of the source of acid that produces the best characteristics of effervescent granules of *kenikir* leaf extract is the composition of citric acid and tartaric acid as much as 75%: 25% with the characteristics of a specific gravity of 0.41g/ml, bulk density 0.72gr/ml, flow rate 12.39gr/ seconds, dissolving time 137 seconds, foam volume 8%, pH 6.32 and has antioxidant activity of 21.37%.

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