Nata de Curcuma

by Har Woko

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Conference Paper *in* AIP Conference Proceedings · January 2023

DOI: 10.1063/5.0106928

Harwoko Harwoko
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23e as: AIP Conference Proceedings **2586**, 030001 (2023); https://doi.org/10.1063/5.0106928
Published Online: 24 January 2023

Annisa Auliya Rahmah, Atika Putri, Felicia Ivena, et al.

Formulation and Evaluation of "Nata de Curcuma"

Annisa Auliya Rahmah, Atika Putri, Felicia Ivena, Monica Ramadhanti, Harwoko Harwoko[a)]

Department of Pharmacy, Faculty of Health Sciences, Universitas Jenderal Soedirman, Jl. dr. Soeparno, Karangwangkal, Purwokerto, 53123 Indonesia

[a)] Corresponding author: harwoko@unsoed.ac.id

Abstract. Turmeric (*Curcuma longa* L.), a well-known spice, often used as an Indonesian traditional medicine or "jamu" ingredient which possesses immunostimulant effect as it contains curcumin. During the COVID-19 outbreak, the consumption of "jamu" tends to increase recently. However, the millennial consumers as one of the target markets do not like "jamu" due to its bitter taste. Accordingly, turmeric should be formulated as nata, namely "Nata de Curcuma" to become "friendly consumed". The study aimed to formulate nata obtained from the water juice of turmeric, to evaluate the physical properties, to identify the curcuminoids, and to test the hedonic. Four formulae of "Nata de Curcuma" consisting of turmeric, coconut water, sucrose, ammonium sulphate (ZA), vinegar, and those fermented with Acetobacter xylinum as a starter. Subsequently, the physical properties of nata including organoleptic, thickness, water content, and fiber content were evaluated. Moreover, curcuminoids in this formula were identified by performing thin layer chromatograph (TLC) method. The best formula of "Nata de Curcuma" contains the mixture of water juice of turmeric and coconut water (1:2) added by 30 g of sugar, 3 g of ZA, and 60 mL of starter. This formula exhibited the thickness of 1 cm and the diameter of 3 cm after 28-days-incubation. The organoleptic properties showed that yellow with the mixed odor of fresh turmeric and slightly coconut, a chewy texture with the specific taste of fresh turmeric but bitterless. The water content of this nata was 95.3% while the fiber content was 4.48% and it contained curcumin evident by the TLC profile. The hedonic test showed that the panelists didn't like the smell of nata due to its smell like turmeric, but they really liked the taste of nata as the unpleasant taste of turmeric was covered. Turmeric was able to be formulated into "Nata de Curcuma" which has the potential as functional food for immunostimulant.

INTRODUCTION

A new strain of corona virus or Severe Acute Respiratory Syndrome Corona Virus (SARS CoV-2) is a virus that attacks the respiratory system resulting respiratory disorders, acute pneumonia, and even death [1]. According to [2], as of September 18th, 2021, in Indonesia there were 4.118.529 positive cases confirmed, with 140.323 deaths. One of the government programs to reduce the spread of this virus by establishing the Task Force of COVID-19 circular No 16th, 2021 and by applying the 6M health protocol included wearing a mask, washing hands with soap in running water, keeping distance, staying away from the crowd, reducing mobility, and avoiding eating together. Furthermore, one of the efforts to prevent COVID-19 disease targeting to the society can be implemented by increasing personal immunity. COVID-19 is curable and a self-limiting disease. The healing of the body is affected by the individual's immune system. Individual immunities can be stimulated through foods and nutritious beverages consumption, for instance by consuming traditional spices as an immune booster.

At the beginning of the pandemic, Indonesian people also use traditional medicine by dispensing corona "jamu" which is assumed to prevent from the virus exposure. "Jamu" is an Indonesian traditional medicine that have been used over the generations for centuries in order to promote health and to treat diseases [3]. The main ingredient in the formula of corona "jamu" is turmeric rhizome which contains the essential oils and curcuminoids. Curcuminoids consists of curcumin (77%), demethoxycurcumin (DMC; 17%), and bisdemethoxycurcumin (BDMC; 3%). Curcuminoids is particularly used for remedies or food supplement [4] with various therapeutic effects, including antioxidant, antibacterial, antiviral, platelet aggregation inhibitor, antidiabetes, and anticancer [5–8], as well as

The Third International Symposium on Food and Agrobiodiversity (ISFA 2021)
AIP Conf. Proc. 2586, 030001-1–030001-8; https://doi.org/10.1063/5.0106928
Published by AIP Publishing. 978-0-7354-4231-3/$30.00

antiinflammation [9]. Based on in silico study, cyclocurcumin and curcumin in turmeric are significantly active against COVID-19 by inhibiting the main protease enzyme of SARS CoV-2 [10]. Curcumin also possesses immunostimulant effect by enhancing the proliferation of lymphocytes, thus increasing the immune system against infection [11].

Recently, immunomodulator is needed to prevent from viral infections, however people are unlike herbs or traditional medicines due to turmeric's bad taste, thereof an innovative formulation is created in the form of Nata de Curcuma. Nata is a food product obtained through a fermentation process with a gelatinous shape and a chewy texture. Nata is produced by Acetobacter xylinum which can produce acetic acid and form thick polycles on the surface of the medium, which the thick polycles are called nata [12]. This study aimed to evaluate the physical properties and to identify the curcuminoids in Nata de Curcuma, as well as to carry out the hedonic test.

MATERIAL AND METHODS

Materials

Acetobacter xylinum, fresh turmeric, aquadest, coconut water, sugar, ammonium sulphate (ZA), buffer at pH 4 and 7, vinegar, Thin Layer Chromatography (TLC) plate of silica gel 60 F_{254}, chloroform, 96% ethanol, glacial acetic acid, and capillary tube.

Formulation of Nata de Curcuma

Preparation of a Starter Stock A. xylinum

The starter stock media was made by boiling 2 liters of coconut water until it boils, then added 200 g of sugar and 2 g of ZA, then wait until the media cooled down to room temperature (20-25°C). The media added by 20 mL of vinegar and 350 mL of main starter, then put into 2 equally glass bottles (600 mL). Covered using clip paper and tied with rubber, incubated at room temperature. After five days at the top, new nata fibers will be formed and the liquid can be used as a starter.

Preparation of Turmeric Juice

Fresh turmerics were sorted to separate the turmeric with dirt or other impurities, washed thoroughly with running water then drained and dried. Turmeric was peeled to be separated from the skin and cut into the small pieces. Turmeric juice was made using 2 types of water, distilled water, and coconut water. 50 g of turmeric in the small pieces added by 95 mL of distilled water/coconut water (for a glass bottle 200 mL), could be multiplied according to the required amount, then pulverizing using a blender. After being powder, turmeric juice filtered and squeezed using a cloth until no fibers are left behind.

Preparation of Fermentation Media and Inoculation of A. xylinum

Fermentation media was formulated by modifying the amount of added sugar, ammonium sulphate, and starter to obtain the best formulation.

TABLE 1. Formula of Nata de Curcuma

Materials	F1	F2	F3	F4	F5
Turmeric	50 g	50 g	50 g	50 g	50 g
Aquadest / coconut water	95 mL	95 mL	95 mL	95 mL	95 mL
Sugar	20 g	20 g	30 g	30 g	0,95 g
Amonium sulphate	1,5 g	3 g	1,5 g	3 g	0,475 g
Vinegar*	15 mL	15 mL	15 mL	15 mL	15 mL
***A. xylinum* Starter**	30, 45, 60 mL	30, 45, 60 mL	30, 45, 60 mL	30, 45, 60 mL	30, 45, 60 mL

*added until pH ± 3

A total of 285 mL of turmeric juice was heated to a boil, then it was added by sugar and ZA according to the formula (amount of sugar and ZA multiplied by 3 for 3 formulae, for example F1 made by 3 formulas ect.). The medium was cooled until it reached room temperature, then gradually added 15 mL of vinegar (until pH ± 3) while measuring the pH using a calibrated pH meter, then put in 3 equal glass bottles. 30, 45, and 60 mL of starter were added to each bottle. The bottle was closed and shaken to mix the media with the starter, the bottle cap opened, and it was replaced by clip paper and tied with rubber. It was then incubated at room temperature for 14-28 days until nata was formed. The same treatment was repeated for all formulae.

Evaluation of Nata

Organoleptic Test

The nata was washed with running water several times until sour smell disappeared. Subsequently, organoleptic tests were carried out by determining the color, taste, aroma, and texture of Nata de Curcuma.

Identification of Curcuminoids

Curcuminoids were identified by using TLC method. The stationary phase used was silica gel 60 F_{254}. Silica gel was cut into a size of 10 x 2 cm, lined at each end at a length of 10 cm. The silica plate was heated in an oven at 110°C for 15 minutes. The mobile phase consisting of chloroform:ethanol:glacial acetic acid (94:5:1), put into the TLC chamber, and saturated for 15 minutes. Test the saturation of the chamber by placing filter paper at the top of the chamber without touching the wall or seeing that there was condensation in the chamber. After the mobile phase was saturated, the Nata de Curcuma juice, curcuminoids standard, and turmeric juice were spotted using a capillary tube over the silica gel approximately 0.5 µl below the boundary line and mark the spots. Inserted TLC plate into the chamber with the spot marked below, left for a few minutes until the solution rises to the upper limit mark. Lift and dry the TLC plate, measure the distance from the start line to the end of the moving spots under the UV lamp at the wavelengths of 254 and 366 nm, then the retardation factor (Rf) value was calculated by formula (1).

$$Rf = \frac{\text{distance traveled by solute}}{\text{distance traveled by solvent}} \quad [13] \quad (1)$$

Determination of Water Content

Determination of water content was performed by gravimetric method. First, the porcelain dish was heated in an oven at 100°C for 15 minutes then cooled and recorded the weight as Wo. Several samples were weighed using a cooled cup and recorded as Ws. The sample was heated in oven at 105°C for one hour. Waited for the cup to cool, weighed the weight of the cup containing residue and recorded as Wr. Calculated the water content using the formula (2):

$$\text{Water content} = \frac{(\text{Wr-Wo})}{(\text{Ws-Wo})} \times 100\% \quad [14] \quad (2)$$

Determination of Fiber Content

Weighed Nata de Curcuma that had been mashed as much as 4 g and dissolved in 15 mL of 96% ethanol in an Erlenmeyer and stirred for 30 seconds. The sample solution stood for 15 minutes, then filtered by filter paper which has been weighed early. The precipitate remaining in the Erlenmeyer was dissolved with 45 mL of 96% ethanol and filtered again. The residue on the filter paper was placed in an aluminum dish and baked in the oven until the residue was dry. After that, put in a desiccator for 15 minutes. The residue and filter paper were weighed again. The dry residue was dissolved in 50 mL of 1.25% sulfuric acid and heated in a water bath for 30 minutes at 60°C. Subsequently, 50 mL of 3.25% NaOH was added and reheated in a water bath for 30 minutes at the same temperature. After 30 minutes, the solution was filtered using filter paper which had been constant. The residue was washed with 25 mL of 1.25% concentrated sulfuric acid, 25 mL of hot distilled water, and 25 mL of 96% ethanol. Then, the residue is placed in an aluminum dish and will be baked until the weight is constant. The residue was put in a desiccator and the constant weight was calculated. At the end, the fiber content can be calculated by formula (3).

$$\% \, Fiber \; content = \frac{\text{Residue}}{\text{Sample}} \text{x} 100\% \qquad [14] \qquad (3)$$

Hedonic Test

Nata de Curcuma freshly harvested was checked several times until the sour smell disappeared. Sugar was added into the water until the sugar water shrink. The cooked nata was packed in a small cup as a sample. The hedonic test was conducted on 30 panelists in the age ranging from 19 to 22 years with the condition that they have already consumed turmeric processed herbal medicine. Panelists were given a sample of processed nata, followed by filling out the form to compare Nata de Curcuma with fresh turmeric juice, testing their color, aroma, and taste.

Data Analysis

Descriptive analysis was performed towards the organoleptic parameters, TLC profile, water and fiber content, of those will be compared with the literature.

RESULT AND DISCUSSION

Nata de Curcuma is one of the innovative products of nata containing curcuminoids obtained from a turmeric spice, hereinafter it can be developed as nutraceuticals (Fig. 1). Nutraceuticals are some of the ingredients that can be considered as food or part of a meal and have health and medical benefits, usually packaged as a preparation [15]. Curcumin in turmeric has a function as a immunostimulant, which curcumin reported to increase lymphocyte proliferation so that it can stimulate the body immune system fight infection [11].

FIGURE 1. Result of Nata de Curcuma

Formulation

The formation of nata in turmeric water media took longer time than coconut water media. Coconut water is a very suitable medium for making nata de coco, because it contains simple sugars [14]. Coconut water contains some nutrients such as carbon which is needed in the production of nata de coco. The presence of sucrose in coconut water is utilized by *A. xylinum* as an energy source, as well as a carbon source to form metabolites such as cellulose which forms nata de coco [16,17]. Coconut water contains sucrose while turmeric does not. This is a factor why the production time of nata de curcuma longer than nata de coco. In the fermentation process, the first step that occurs is the hydrolysis of sucrose into glucose and fructose. Therefore, in the production of nata de coco that contains sucrose, the fermentation process will be faster compared to nata from turmeric.

In this study, we found that turmeric water juice can be used as a fermentation medium of nata de curcuma's formulation. In addition, it was also found that the best formula was determined by the amount of nata formed, after 28 days of incubation there was nata with 1 cm of thickness and 3 cm of diameter in the mixed media of coconut water and turmeric juice with 30 g of sugar, 3 g of ZA, and 60 mL of starter.

Organoleptic Properties

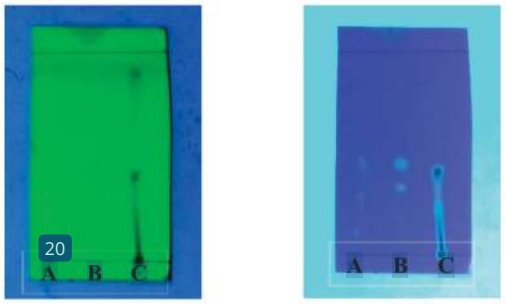
Organoleptic test is a test using human senses as the main tool for measuring and evaluating products or samples in order to control the quality of nata. This test was carried out by detecting the color, aroma, and taste of nata. Freshly Nata de Curcuma (Fig. 1) has a yellow color like egg yolk, aroma like fresh turmeric with a slight coconut smell, dense and chewy texture, and has a distinctive taste of turmeric but bitterness like fresh turmeric (Table 2).

TABLE 2. Organoleptic Properties of Nata de Curcuma

Organoleptic	Nata Standard	Control Formula	Formula of Nata de Curcuma (Media turmeric & coconut water)
Color	White nata	White nata	Yellow like an egg yolk
Aroma	No smell	Aromatic from fermentation process	Mixed odor of fresh turmeric and slightly coconut
Texture	Solid	Solid	Chewy
Taste	Not sour	Sour	Bitterness with specific taste of fresh turmeric

Identification of Curcuminoids

Curcuminoids in nata de curcuma was identified by performing thin layer chromatography (TLC) technique. The stationary phase used was silica gel 60 F_{254}, while chloroform:ethanol:glacial acetic acid (94:5:1) used as the mobile phase. After elution, it was detected that nata de curcuma dissolved in water showed identical spots with curcumin standard, thus nata de curcuma containing curcumin. The Rf value of nata was identical with curcumin standard which appeared at Rf value of 0.50. Meanwhile, a fresh turmeric juice as a raw material possessed the Rf value of 0.46 for curcumin, suggesting that curcumin in turmeric was still found in nata de curcuma.



(I) (II)

FIGURE 2. TLC profile observed under the UV_{254} (I) and UV_{366} (II) of sampel A = Nata de Curcuma, B = Curcuminoids standard, C = Fresh turmeric juice

Water Content

Water content was determined by the gravimetric method. The water content obtained from the best formula with 28 days of fermentation was 95.3%. According to the literature, the standard water content in nata is not less than 85%. When *A. xylinum* added to a medium containing carbohydrate (sugars) will produce a polysaccharide known as extracellular cellulose and can undergo further oxidation, which is capable of oxidizing acetic acid to carbon dioxide and water. In this case, the longer the fermentation time, the more water is trapped in the nata layer and resulting in the higher water content [18].

(a) (b)

FIGURE 3. Nata before (a) and after (b) determination of the water content

Fiber Content

The results of the fiber content of Nata de Curcuma from fermentation for 28 days was 4.48%. This value was in accordance with the nata standard, which stated that the fiber content of nata should not exceed 4.50%. The fiber formed is the result of sugar reform in the fermentation medium by the activity of *A. xylinum*. The increase in the activity of *A. xylinum* causes the increase the cellulose production. Long fermentation can cause the cellulose secreted by *A. xylinum* to bind strongly to one another to form layers that continue to thicken. The more *A. xylinum* excretion, the higher the crude fiber produced from the fermentation process. Moreover, the longer the fermentation, the higher the fiber content of nata. This fiber content is important for the human digestive process. The higher the fiber content in nata, the better the nata product formed is used as a food additive [18,19].

The Hedonic Test

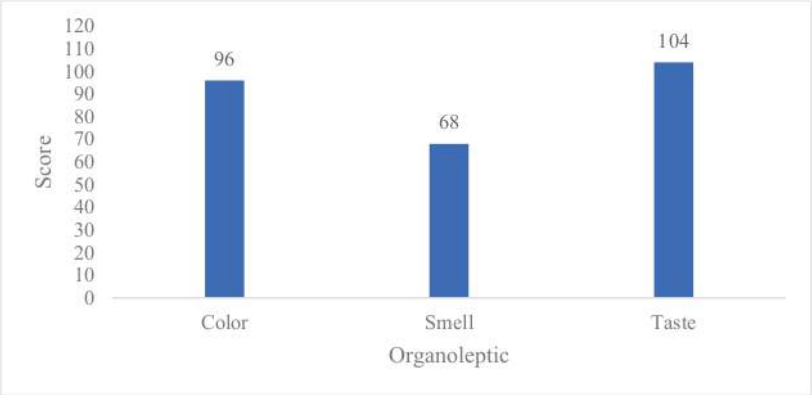


FIGURE 4. Hedonic Test Parameters

Hedonic test has been conducted on 30 panelists, and the results obtained 96 points on color, 68 points on smell, and 104 points on taste. Taken together, the panelists like the color of nata, but do not like the smell of nata, and really like the taste of nata. As stated by previous researcher [20-22] that was found that traditional food stuff commonly provides unpleasant flavor. Therefore, panelist didn't like the smell of nata due to the existing of turmeric smell, but panelist really liked the taste of nata where the unpleasant taste of turmeric was covered.

CONCLUSION

Formula 4 (F4) is the best formula of "Nata de Curcuma" containing the mixture of water juice of turmeric and coconut water (1:2) added with 30 g of sugar, 3 g of ZA, and 60 mL of starter. This formula possessed the thickness of 1 cm and the diameter of 3 cm after 28-day-incubation. The organoleptic properties revealed yellow with the mixed odor of fresh turmeric and slightly coconut, a chewy texture with the specific taste of fresh turmeric but bitterness. The water content of this nata was 95.3%, while the fiber content was 4.48% and it contained curcumin as evident by the TLC profile. From the hedonic test the panelists did not like the smell of nata because it still smelled of turmeric, but they really liked the taste of nata. Thus, turmeric is able to be formulated into "Nata de Curcuma" which has the potential as immunomodulator functional food.

ACKNOWLEDGMENTS

The authors thank the Directorate General of Higher Education, Ministry of Education, Culture, Research and Technology for funding this program (PKM-RE 2021).

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