

The Effect of Dryer on Antioxidant Levels and Antibacterial Power of Turmeric Juice Nanocapsules Powder

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Abstract— This study aims to find a drying tool from turmeric juice nanocapsules (TJN) to get the best powder product. Due to the nature of liquid TJN products, it is difficult to apply as a natural additive feed in feed factories, it needs a special place for storage and has a short shelf life. Experimental research has been carried out in 3 drying treatments, namely with tools: 1_spray-dryer; 2_cabinetdryer; 3_food-dehydrator. Each treatment was carried out 3 times. After the drying process, the powder samples (TJN-1), TJN-2 and TJN-3 are characterized in: Their antioxidant and antibacterial properties. The results of Anova showed a significant difference (P<0.05) between treatments, successively the results of drying with 1)_spray-dryer (TJN-1); 2) _cabinet-dryer (TJN-2); 3) _fooddehydrator (TJN-3) for results Chemical characteristics: a). Dry *matter content(%)*: 1). 92.59±0.020.; 2) 89.18±0.030, 3) 92.03±0.050; b). Curcumin content (%): 1) 2.83±0.016; 2) 2.25±0.060, 3) 1.97±0.010; c). Antioxidant levels (%RSA): 1) 81.63±0.650, 2) 85.19±0.540, 3) 84.77±0.001. Antibacterial power of inhibitory diameter (mm) in E. coli at a dose of 125 mg/ml: 1). 6.50±0.40, 2). 11.10±0.30, 3). 5.95±0.05 and in Subtilis Bacteria 1). 9.05±0.45, 2). 5.55±0.35, 3). 5.70±0.30. From the characteristic data obtained, it was concluded that the best TJN dryer is a Cabinet dryer with an antioxidant level of 85.19±0.54 %RSA and antibacterial (inhibition diameter in E. coli) of 11.10±0.30 mm at a dose of $125 \,\mu\text{g/ml}$ which is better than other dryers.

Keywords— Antioxidant; Curcumin; Dryer; Feed_Additives; Turmeric_Juice_Nanocapsule.

I. INTRODUCTION

Facts in the field Examination of veterinary drug antibiotic residues also occurred abroad, including the results of a study in Nigeria showing that 59 out of 178 broilers (33.1%) were found to have veterinary drug residues in chicken tissue and oxytetracycline is the most widely used drug. Research conducted in Croatia found that the level of tetracycline in meat was 1.62 µg/kg (1). The presence of antibiotics in animal tissues will be consumed by humans and if consumed continuously will trigger resistance. Therefore, antibiotic resistance is considered by the World Health Organization (WHO) as one of the major global threats to public health. The WHO has published a list of bacteria that are urgently needed new antibiotics, to treat them and fight resistance (2). Bacteria have a very high rate of random genetic mutations (and they reproduce very quickly, which further favors the emergence of mutations). Certain mutations can develop defense

mechanisms in bacteria, allowing them to fight off antibiotics. In addition, bacteria are constantly exchanging genetic material with each other, and can therefore transfer the genetic material that supports resistance, spreading it rapidly. This is known as antibiotic resistance. The first ban on the use of antibiotics occurred in Europe in 2006, European regulatory changes have led to a drastic reduction, or even elimination, in the use of antibiotics in animal feed. Antibiotics sold in medicinal feeds in France have fallen from about 400 tons in 2011 to 21 tons in 2022, a reduction of 95% (3) Through Ministerial Regulation No. 14/2017 concerning Classification of Veterinary Drugs, since January 1, 2018 the Government of Indonesia has prohibited the use of Antibiotic Growth Promoters (AGPs) in feed. This prohibition is also strengthened by Ministerial Regulation No. 22/2017 (4). concerning Feed Registration and Circulation, which requires a declaration not to use AGP in the formula of feed produced for producers who will register feed. The ban on the use of AGP makes all feed producers try to find alternatives a commensurate replacement for AGP. This aims to keep chicken productivity optimal. Currently, there are many available and circulating alternatives to AGP on the market such as organic acids, probiotics, prebiotics, synbiotics, phytobiotics, enzymes, etc. Circular Letter of the Director General No. TN 260/1994 there are 19 antibiotics that include AGP: among others virginiamycin, zinc bacitrasin, etc. Until now, there has been no replacement product that is cheaper and better than AGP. It has successfully innovated a natural AGP product from turmeric extract nanoencapsulated into chitosan cross-linked with STPP. There are 3 types of this product, namely Nanoturmeric powder (5). and Liquid Nanoturmeric (turmeric filtrate nanocapsules (6). and turmeric juice nanocapsules(7). All three have been proven to increase the quantity and quality of poultry meat production. However, there are still obstacles to its wider use.

The research is based on the nature of liquid turmeric juice nanocapsule (TJN) products that are difficult to apply as natural feeds additive in feed factories, need a special place for storage and have a short shelf life. The problems faced include the need for cold temperature storage such as in the refrigerator or freezer for a long use period. Of course, this is less practical considering that not all farmers have a

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refrigerator. Besides that, in its liquid form, it requires a high level (2-4%) to produce optimal growth. The storage requires a large or larger space, rather than the powder form. The efforts that have been made, including 1). Made turmeric nanocapsules of ethanol extract (product-1) in the form of a powder that has been granted a patent with the IDP000066541 the price of the product is very expensive, much more expensive than the synthetic antibiotic bacytracin [5]. Furthermore (product-1) above in Innovate/improve so that the product price can be affordable, namely making a Turmeric Filtrat Nanocapsule (product-2) which has also been granted with IDP000067179, but this product-2 is also a bit complicated in the manufacturing process because a filtration process is needed in the middle of the mixing process with a mixer (6). Finally, the process was improved again without filtration or filtration at all in the end-process and produced product-3, namely turmeric juice nanocapsule / TJN (which was used in this study). TJN patent status in the process of substantive examination with registration number P22201906691 (7). TJN has been applied in poultry feed / drinking water (Ducks and KUB_Chickens), and also as a curing ingredient in the processing of poultry meat into shreds, nuggets, sausages and meatballs as well as soft bone chicken.

The gap or obstacle that is still felt in the use of TJN is: having been offered cooperation with the feed mill industry, they have not agreed because they feel that liquid products are still difficult to mix with feed because they are not compatible with existing mixer machines. Besides that, the liquid form requires a container and cold temperature for storage. Liquid products are prone to microbial contamination, the results of the TJN author's research are still of good quality if stored for 1 month in a refrigerator at a temperature of 4°C (8). So the research aims to find TJN dryers in various ways to get dry powder products with good antioxidant and antibacterial content and affordable prices. The implications of this study will be to produce nanocapsule powder products jus_kunyit that are high in antioxidant and antibacterial substances at affordable prices. In the future, this best product will be added 3 more types of critical amino acids, namely lysine, methionine and threonine to improve livestock performance. The product in question is NanoKu+ (Nanocapsules Jus Kunyit plus amino acids), as a feed additive that is expected to replace AGP.

II. MATERIALS AND METHODS

Materials, Tools and Methods Used

Turmeric Juice Nanocapsule Ingredients: Turmeric Rhizome (obtained from Beringharjo Market, Yogyakarta-Indonesia), Kitosan (PT. Surindo Biotech, Cirebon-Indonesia), technical STPP (Misonyal-Indonesia brand), Technical citric acid (Gajah brand-Indonesia), drinking water (Le Mineral). Chemical analysis materials: Curcumin, DPPH, Nutrient Agar.

Research Equipment: 1). Equipment for TJN Production: Blender-Mixer Machine capacity 20L, 2880rpm, 1HP), one set of Gas Stove, one set of blanching equipment pan, scale, 2). Laboratory equipment of memmert ovens, analytical scales, micropipettes and glassware, curcumin levels and DPPH- antioxidant tests with UV-Vis spectrometers. Test the color with the NH300 colorimeter.

Method of making feed additives of turmeric juice nanocapsules (7). substitutes for synthetic antibiotics, antioxidants and anti-cholesterol as follows: Fresh turmeric rhizomes 4 kg peeled and blanched with a 0.05% (2 g) citric acid solution and put in boiling water for 5 L for 5 minutes. Put the turmeric rhizome and 5 L of boiled water in a blendermixer for 2x30 minutes, turmeric juice is produced. Encapsulate turmeric juice by mixing 50 g of chitosan that has been dissolved in 4 L of 2.5% citric acid (100 g) into a blender-mixer then mixed/swirled for 30 minutes, cross linkkankan the skin of the chitosan capsules by adding 25 g STPP which has been dissolved in 1 L of aquades/drinking water mixed into a large mixer (TTG device) with a capacity of 20 L for 30 minutes, the result of the product is a liquid preparation turmeric juice nanocapsule with a concentration of 100%. If you want to pack it, strain it with a cloth first.

The study was carried out with a completely randomized design of oneway: TJN_liquid the results of the above manufacturing were weighed @ 1kg as many as 9 containers, then randomly divided into 3 drying treatments, namely with tools: 1 spray-dryer; 2 cabinet-dryer; 3 food-dehydrator, for each treatment was repeated 3x. The data obtained were analyzed for variance if there was a significant difference between the treatments tested with DMRT using SPSS. After the air dries, the dry weight is weighed. The dry air sample was then analyzed: moisture content (Dry-Material/DM), ash content (Organic-Material/OM) using the AOAC (9). Measurement of curcumin levels using the SpectroUV-Vis method (10). The first time a standard curcumin curve was made by making a series of curcumin concentrations of 0, 1, 2, 3, 4 and 5 ppm, then the absorption was marked on a UV-Vis spectrophotometer at a wavelength of 426 nm. After completion, it was continued by making a standard curve of curcumin between absorbance vs concentration so that the equation Y = 0.1964 x + 0.0054 (R2 = 0.9976) was obtained. Sample preparation procedure: 1 gram of sample plus 10 ml of ethanol, filtered then taken 10 microliters to make 10 ml with ethanol, indicated absorption at a wavelength of 426 nm and the concentration of sample curcumin can be obtained by entering the value of x into the equation above. Antioxidant measurement by DPPH method(11); (12). The free radical capture capacity is determined as follows: 0.2 ml sample plus 3.8 ml of 0.1 mM DPPH solution, 1-minute divortex, and incubated at room and room temperature gelap selama 30 menit. Absorbance is noted at λ 517 nm. Blanks (controls) by using ethanol as a substitute for samples. The capture power of free radicals is expressed in percent (%) RSA = % Radical Scavenging Activity is the % of DPPH dismissal. Antioxidant activity is calculated by the following formula: % Antioxidant activity = [(Blank Absorption - Sample Absorbance)/ Blank Absorbance] x 100%

Antibacterial tests were carried out using the disc diffusion method (13). The tools used in this study are electric autoclaves (Hirayama), a set of glass tools consisting of petridish, test tubes, tube racks, measuring cups, erlenmayer (Pyrex), incubator (Memmert), calipers (Tricle Brand), ose

needles, Laminar Air Flow (LAF), analytical scales (Ohaus), and vortex. The materials used in this study are turmeric juice nanocapsule powder, E. coli bacteria, aquadest, Nutrient Agar (Oxoid), ethanol, filter paper, ampicillin disk, cotton, rubber. Testing of antibacterial activity on juice-turmeric nanocapsule powder products was carried out by disc diffusion method using paper disc with a diameter of 5 mm. NA media that has been inoculated with test bacteria (E. coli), pasted on disc paper and then samples are inserted using a micropipette of 10 microliters per disk, at each concentration of 0.625, 125, 250, 500, 1000, 2500, and 5000 μ g/ml and then placed. Then it is incubated at 37°C for 24 hours. The observation was made by looking at a clear zone around the paper disc that showed an area of inhibition of bacterial growth. Measurement of bacterial inhibition zones using a caliper. The calculation provision, if there is a large and small diameter of the inhibition zone, the two are added and then divided by half and recorded. Measurements were taken 3 times and taken on average. After the results of chemical and biological analysis are completed, the price of each TJN-1, TJN-2, TJN-3 product per gram of the cheapest DM is evaluated from the high content of antibacterial substances (inhibitory power in bacteria) and antioxidant substances (%RSA).

III. RESULT AND DISCUSSION

This research is an applied research to find the best solution so that Turmeric Juice Nanocapsules (TJN) products can be absorbed by the market more. There are many disadvantages of liquid TJN, for example because of its liquid form, this material is more difficult to use / mix as a component of rations because it is difficult to be homogeneous (sticky). In addition, the design of the mixer machine in the feed factory is indeed prepared to mix solid feed ingredients. So when TJN was offered to supply/replace the Growth Promoter Antibiotics (AGP) that have been used by factories such as zinc-bacitracyn, it was rejected. Its use in traditional farmers is only small as a substitute for AGP, after all, if this TJN is included in the ration, many farmers complain about the hassle, because it must be mixed first before being given either in feed or drinking water. Feed that has been mixed with TJN is liquid, becomes moist and easily moldy. Liquid TJN also needs a container and a refrigeration machine for subsequent storage. Some of these reasons encourage us to carry out the drying process, so that all existing obstacles can have a solution. In order to find a solution to the above problem, the following results of the experiment are displayed in Table 1. Chemical composition of turmeric juice nanocapsules with different dryers and Figure 1. Color embodiment of Turmeric Juice Nanocapsules Characterized using NH 300 colorimeter using L, a and b scales whose determination is based on the hunter color notation system. In Table 1, the results of the analysis of dried powder samples of turmeric juice nanocapsules from drying using different drying tools: 1-Spray dryer, 2-Cabinet dryer, and 3-Food dehydrator. The 3 tools were selected and used at a medium temperature of 50-60°C so that only water evaporates and does not damage the active ingredients in TJN. The most important main active substance of TJN is curcumin and its antioxidant substances.

The curcumin content in TJN powder is: 1.97 - 2.83% and antioxidants: 81.63-85.19%RSA. The use of drvers has a different effect, from table 1 it turns out that the use of a cabinet dryer is able to provide the highest level of antioxidants (85.19%RSA) with an affordable drying equipment rental cost (Rp.2,669.78/g DM) but cheaper with a food dehydrator Rp 2490.89/gDM. This is in accordance with the opinion of Suprihatin et al. (14), stating that there are 11 compounds that have the potential to be antioxidants in turmeric powder based on in silico analysis , namely: Ascorbic acid, Quercetin, β Carotene, Arabinose, Bis Demethoxycurcumin, Demethoxycurcumin, Curcumin, Caffeic acid, Cinnamic acid, Letestuianin A, and Calebin A. In detail, reported that the highest concentration of compounds contained in turmeric rhizome powder is curcumin (C21H20O6) which is 7.798%, while the concentration for curcumin derivative compounds, namely demethoxycurcumin (C20H18O5) and bisdemethoxycurcumin (C19H16O4) is 4.115% and 2.277% (14).

From Table 1, the chemical composition is significantly different (P<0.05) in the content of dry materials, organic materials, curcumin and antioxidants, this is due to the different drying systems of the 3 types of tools used. This is in accordance with the opinion (15) which states that the method of drying turmeric rhizomes affects the content of compounds contained in the rhizomes of turmeric plants and their concentration. Curcumin is able to increase its antioxidant activity by capturing various species of reactive oxygen (ROS) such as superoxide radicals, hydrogen peroxide, and nitric oxide (NO) radicals as well as by inhibiting lipid peroxidation (16). The latter activity is due to increased activity of many antioxidant enzymes, such as Superoxide dismutase (SOD), CAT (catalase), GPx (glutathione peroxidase), and OH-1. Curcumin can also increase GSH/glutathione levels by increasing glutathione transferase and its mRNA. Curcumin can also inhibit ROS-producing enzymes, such as Lipoxygenase/LOX, COX/Cyclooxygenase, and xanthine oxidase. Curcumin is also considered a chain-breaking antioxidant due to its lipophilic properties, potentially acting as a perocyl radical scavenger(17). The potential antioxidant is to be an activator of Nrf2. Nrf2 (Nuclear Related Factor 2) is a transcription factor that binds to a specific repressor, namely Keap1. Nrf2 can be active if it does not bind to Keap1, commercial drugs have been widely developed to activate Nrf2 by inhibiting Keap1. The activation of Nrf2 that does not bind to Keap1 is a representation of the initiation of the synthesis of antioxidant enzymes in an effort to prevent oxidative stress and aging in cells (18).

In the results of the inhibition test of turmeric juice nanocapsule powder on the growth of E. coli bacteria (Table 2), the highest dose of TJN powder $125 \,\mu$ /ml was 11.10 ± 0.300 mm. The results are strong because they are in an obstacle area of 10-20 mm in the strong category (19). This is because turmeric (curcumin) is in an encapsulated position by chitosan which is chemically cross-linked to STPP, forming a stable capsule shell, as illustrated in Figure 2. Curcumin is a less polar compound that is insoluble in water but soluble in alcohol (20). Some biological activity of curcumin has been

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identified, but the high hydrophobic properties of curcumin in the gastrointestinal epithelium are a major weakness of its therapeutic activity and clinical application. It is reported that the curcumin nanosphere (nCur) has a sharp absorption peak around a wavelength of 430 nm and a typical functional group, which is known to be a characteristic of curcumin, and has up to 160 times greater water solubility than curcumin. It is reported that curcumin-containing nanospheres (nCur) increase the motility of gastrointestinal epithelial cells by stimulating F-actin-related cytoskeletal reorganization via the CCP-mediated JNK/NF-kB pathway and may increase enterocyte movement along the cryptocyte-villi axis. The quality of the discs and their antibiotic potency, produced by various manufacturers, must be approved by three agencies, namely the FDA, WHO, and the U.S. Department of Health and Human Services (DNHW) (21). Different levels of antibiotic saturation can be selected by the manufacturer as some discs may contain more than 100% of the stated content to compensate for the loss of activity during disc handling

(21). There are three main international standards for antibiotic potency in discs and not all manufacturers produce according to the same standards. Their specifications have been summarized as follows: FDA specification 67-150%, WHO specification 75-135% and DNHW 90-125% of the concentrations mentioned (21).



Drvers.

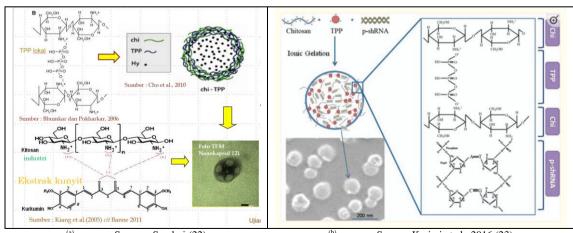
	Drying Treatment using Dryer				
	TJN1-Spray Dryer	TJN2-Cabinet Dryer	TJN3-Food Dehydrator		
Dry Matter (%)*	92,59±0,020 °	$89,18 \pm 0,030^{a}$	$92,03 \pm 0,050^{\rm b}$		
Organic Matter (%)*	$91,99 \pm 0,020^{a}$	$92,92 \pm 0,060^{b}$	93,38±0,020°		
Curcumin (%)*	$2,83 \pm 0,016^{\circ}$	$2,25 \pm 0,060^{b}$	$1,97 \pm 0,010^{a}$		
Anti-Oxidant (%RSA)*	$81,63 \pm 0,650^{a}$	85,19 ± 0,540°	$84,77 \pm 0,000^{b}$		

With any multi same row with anterent	superscript uniter significantly (1 <0.05).
TJN = Turmeric Juice Nanocapsule	e, RSA=Radical Scavenging Activity.

TABLE 2. Inhibition diame	ter of turmeric	juice nanc	ocapsules [powder in	E.coli b	acteria (m	ım)

Dose TJN (µg/ml)	TJN 1-Spray Dryer ¹	TJN 2-Cabinet Dryer ¹	TJN 3-Food Dehydrator ¹
00,00	6.00±0.000 ^b	8.20±0.600 ^a	5.95±0.250 ^a
62,50	6.10±0.100 ^b	9.45±0.250 ^b	6.10±0.100 ^a
125,00	6.50±0.400°	11.10±0.300°	5.95±0.050 ^a
250,00	6.05±0.050 ^b	10.05±1.050bc	6.10±0.400 ^a
500,00	6.10±0.100 ^b	$8.95{\pm}0.050^{ab}$	6.60±0.000 ^b
1000,00	6.13±0.058 ^b	9.60±0.500 ^b	7.05±0.050°
2500.00	5.70±0.100 ^a	9.65 ± 1.050^{b}	6.90±0.200 ^{bc}
5000.00	5.60±0.200 ^a	7.90 ± 0.700^{a}	6.65±0.150 ^b
Means ²	6.023±0.301 ^a	9.363 ±1.119°	6.413±0.451 ^b
Control positive Ampicillin (10 µg/disc)	12.35±3.85	8.3±0.50	11.8±0.80

¹Means in the same column with different superscript differ significantly (P<0.05), 2Means in the same row with different superscript differ significantly (P<0.05). TJN= turmeric juice nanocapsules.



Source : Sundari (22) Source: Karimi et al., 2016 (23) Figure 2. Illustration of the chemical bond between the C=O⁼ group of turmeric binding to the NH3+ group of chitosan, and chitosan also binding to PO4= (forming a strong capsule shell cross-link).

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IV. CONCLUSION

Drying with a cabinet dryer is the best choice for drying turmeric juice nanocapsules into powder, antioxidant content of 85.19% RSA, and antibacterial power in E.coli (inhibition diameter 11.10 mm, at a dose of 125 mg/ml).

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