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Microencapsulation of *Andrographis paniculata* extract using crystallizer at a different agitation speed.

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The covid-19 pandemic has made the influence of functional food even stronger. Sambiloto (*Andrographis paniculata*) is a local herb called "the king of bitters" because of its bitter taste. It has bioactive compounds which function as an immunostimulant. Microencapsulation of *Andrographis paniculata* extract can extend its shelf life and maintain its bioactive compound. Microencapsulation is conducted by crystallization method with sugar as an encapsulating agent. This study was aimed to determine the effect of the agitation speed of crystallizer and the addition of *Andrographis paniculata* extract on the physicochemical characteristics of microencapsulated powder. Crystallization was conducted at 100 °C. This study used three concentrations of *Andrographis paniculata* extract (0.5%, 1%, 1.5% w/w) and three agitation speed of crystallizer (60 rpm, 80 rpm,100 rpm). The results showed that increasing agitation speed contributed to increase yield value and to decrease crystallization time and dissolving time. Increasing *Andrographis paniculata* extract affected on the increase of antioxidant activity. The optimum process condition was achieved at 100 rpm with a concentration of 1,5%, which resulted on crystallization time (110 minutes), moisture content (1.32 %), ash content (1,05 %). dissolving time (70 second), and antioxidant activity (59.50%).

Keywords : microencapsulation, *Andrographis paniculata*, crystallization

1. Introduction

Functional food is a food product that contains particular nutrients that are beneficial to health (Khan et al., 2013). The role of functional food is becoming increasingly in demand during this pandemic. In the midst of the rampant addition of active cases of Covid-19 in Indonesia, the best thing that can be done is to maintain the body's immunity by increasing the consumption of antioxidant compounds. This is closely related to the role of antioxidant compounds in maintaining the body's immunity. This group of compounds can capture free radical molecules and reactive oxygen species to inhibit the onset of oxidative reactions that cause various degenerative diseases (Adawiah et al., 2015). Nevertheless, the consumption of synthetic antioxidants can cause side effects, so the consumption of antioxidants from natural ingredients is more recommended to increase the body's immunity (Sayuti & Yenrina, 2015). Research conducted by Mrityunjaya et al. (2020), shows that many nutritional supplements from several spices can reduce the risk of viral infections by enhance the body's immune system response.

One of the natural ingredients that are a source of antioxidants is the plant, namely *Andrographis paniculata*. Its leaves, is often used as an immunostimulant, diabetes, and others (Maity et al., 2019). This medicinal plant is believed to have the potential to be an inhibitor covid -19 (Sukardiman et al., 2020). One of the main compounds in *Andrographis paniculata* plants is Andrografolid ($C_{20}H_{30}O_5$). which gives bitter taste characteristics to *Andrographis paniculata* (Thakur et al., 2015).

Another spice ingredient that has been widely known for its antioxidant compound content is Ginger. The ginger plant (*Zingiber officinale*.) is a multifunctional cash spice plant. The rhizome of this plant can be used for various purposes, including cooking spices, medicines, and flavor enhancers (Firdausni et al., 2017). Ginger rhizomes have many functions in traditional medicine, including as a remedy for colds, headaches and appetite-enhancing stimulants (Srinivasan, 2017). Ginger contains antioxidant compounds consisting of gingerol and shogaol (Embuscado, 2015).

Andrographis paniculata extract can be processed into a health drink. In this study, this extract was combined with ginger extract and sugar through the crystallization method to determine the optimal influence and concentration on the combination of *Andrographis paniculata* extract and instant ginger powder drink. Herbal powder drinks are usually processed using crystallization. The crystallization process uses a crystallizer as a pan for heating the solution and a stirrer driven by a hydraulic pump that uses electricity as its main power source.

One factor determining the crystal formation rate is the agitation speed, which involves heating the solution/extract to a saturated state and then stirring. The proper agitation speed can result in optimal performance against the crystallization process of powdered beverages. The purpose of this study was to determine the effect of the treatment of agitation speed and the proportion of the addition of *Andrographis paniculata* herbal extract on the physicochemical characteristics of instant ginger powder drink products processed by the crystallization method.

2. Methods

2.1. *Andrographis paniculata* Leaf Extraction

The *Andrographis paniculata* leaves were sorted and washed with running water to remove the dirt attached to the herbs, such as dust, soil, and other impurities. (Wardatun, 2011). Then the *Andrographis paniculata* leaf was weighed as much as 500 grams and dissolved in distilled water with a ratio of 1:3. The material was crushed and homogenized using a blender. The solution was boiled for 15 minutes while the temperature of the solution was controlled (below 70^{o}C), then cooled, and the filtrate was filtered using a juice extractor. (Haryanto, 2018).

2.2.Ginger Rhizome Extraction

Ginger rhizomes were sorted, washed, and cut into small pieces. Then, the ginger was weighed as much as 500 grams and then blended until homogeneous with the addition of 1500 ml of water (Desnita et al., 2019). The ingredients were boiled for 15 minutes and then filtered to separate the sediments and the filtrate (Haryanto, 2018). The filtrate was put into a container and decanted for 2 hours (Desnita et al., 2019).

2.3.Crystallization of Instant Ginger Powder Drink

The powder drink crystallization process was adapted from the method of Haryanto (2018) and Musita (2019). Five hundred grams of ginger extract filtrate was put into the crystallizer. After that, bitter leaf extract was added with 3 different concentration treatments (0.5%, 1%, 1.5% w/w). Then 250 grams of sugar is added to the solution. The crystallizer machine is turned on at 75oC with three variations of agitation/stirring speed (60 rpm, 80 rpm, and 100 rpm) (Khairunisa et al., 2019). The solution is heated and stirred until crystal fibres are formed. After obtaining crystals, they were then cooled. The rate of crystal formation was measured using a timer, where measurements were carried out qualitatively with visual observations to form crystal fibres.

The crystals formed were cooled at room temperature and then mashed using a dry blender. The crushed crystals were put into a shaker and sieved through a 100 mesh sieve to obtain the same powder size. (Desnita et al., 2019). Then the drink powder is put into the packaging and tightly closed (Haryanto, 2018).

3. Physical and Chemical Analysis of Instant Powder Drink

Yield Value

The yield test method was adapted from Khairunisa et al., (2019). The weight of the crystallized powder drink was weighed using an analytical balance and then calculated the percent yield formed. Yield levels of powdered beverage products were calculated using the following formula:

$$Yield\ Value\ (\%) = \frac{Output\ Weight}{Input\ Weight}\ x\ 100\%$$

Moisture Content Analysis (*Wet Basis*)

Moisture content analysis was conducted using the oven method adapted from Meldayanoor et al., (2019). The empty cup was placed in the oven at 105°C for 1 hour then cooled in a desiccator for 15 minutes and weighed (W1). Samples of powdered drink that have been weighed with a weight of 5 grams (W) were put in a cup, and dried in an oven at a temperature of 105°C for 3 hours. The sample in the cup was cooled in a desiccator for 15 minutes and then weighed to a constant weight (W2). The moisture content in the product was calculated by the following formula:

$$Water\ Content\ (\%) = (\frac{(W1 + W) - W2}{W})x100\%$$

Ash Content Analysis

Ash content was analyzed by using the kiln method adapted from Meldayanoor et al., (2019). Empty crucibles were put in a furnace at a temperature of 550°C for one hour, then cooled in a desiccator for 15 minutes and weighed (W1). The sample was weighed for one gram (W) and put in an empty crucible, then put in a furnace at a temperature of 550°C for 4 hours. After that, the sample was cooled in a desiccator for 15 minutes and then weighed (W2). The following formula calculated the ash content in the product:

$$Ash\ Content\ (\%) = (\frac{(W1+W)-W2}{W})x100\%$$

Dissolving Time Analysis

Testing the solubility of the product using a method adapted from Aliyah (2019). A total of 10 grams of powdered drink samples were dissolved in 100 ml of water at a speed of

400 rpm. Solubility was calculated by measuring the dissolving time of the powder (s) when dissolved in water.

pH Analysis

Instant powder drink pH test adapted from the method Wala et al.,(2016). A total of 10 grams of instant ginger powder sample was dissolved in 100 mL of distilled water and then stirred until homogeneous. Prior to use, the pH meter was calibrated with a buffer solution of pH 4 and 7. The electrodes of the pH meter were immersed in a solution of instant ginger drink until a stable reading was obtained.

Antioxidant Activity Analysis

Analysis of the antioxidant activity of powdered beverage products using a UV-Vis spectrophotometer with the DPPH method adapted from Molyneux from Widyani et al. (2019) and (Hussein et al., 2017). The sample was weighed as much as 0.5 grams, dissolved in 5 ml of 96% ethanol, and immersed in a UC-10SD ultrasonic cleaner water bath for 15 minutes. The sample was allowed to stand for 2 hours to form a precipitate and residue. A total of 0.1 ml of the precipitated sample was taken and dissolved with 3.9 ml DPPH solution. Then the test tube containing the sample was wrapped in aluminium foil and kept in the dark room for 30 minutes. After being stored for 30 minutes, the sample was vortexed, and its absorbance was measured using UV-Vis spectrophotometry with a wavelength of 517 nm. The blank solution was made with 0.1 ml of ethanol and 3.9 ml of DPPH solution, which had been left for 30 minutes. The test tube containing the blank solution was wrapped in aluminium foil for 30 minutes to prevent damage. Antioxidant activity can be calculated using the formula:

$$Antioxidant\ Activity(\%) = \left(\frac{control\ absorbance - sampel\ absorbance}{control\ absorbance}\right) x100\%$$

Data Analysis

Based on the research data, continued data processing using Microsoft Excel in the form of linear graphs. The analysis of crystallization time and dissolving time were carried out using SPSS (Statistical Package for The Social Science). The data will be analyzed by statistical test two way ANOVA with 95% confidence level. Then to find out how much the difference between each treatment is, it is continued with the Duncan Multiple Range Test (DMRT).

3. Results and Discussion

3.1. Yield

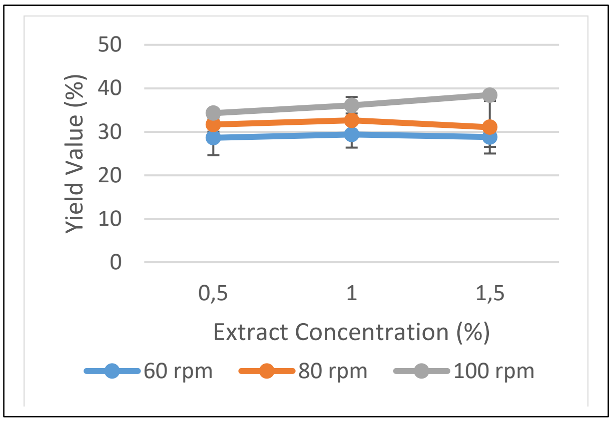

Figure 1. Yield Value

Figure 1 shows the profile of yield on each treatment. From the graph, it can be seen that the yield value is directly proportional to the increase in the speed of agitation used. The faster the speed of agitation used, the greater the resulting yield. This is in accordance with the theory that the higher the speed of stirring will increase the percentage of yield from the crystallization process (Khairunisa et al., 2019).

The crystallized powder with an agitation speed of 100 rpm is the powder with the most homogeneous particle size. This is also supported by Mullin, (2001), who states that the appropriate stirring speed can give crystallization results with a more homogeneous size. Powders obtained from crystallization with agitation speeds of 80 and 100 rpm tend to have a darker color and light brown color compared to powders resulting from crystallization speeds of 60 rpm, with colors tending to be white and brighter. The appearance of the powder colour shows that the heat at the treatment speed of 80 rpm and 100 rpm can be spread evenly. It is in accordance with the theory of Mursalin et al. (2019), which states that stirring is intensive and necessary so that the heat can be evenly spread on the material. Intensive stirring is needed when the solution begins to harden so that the formed crystals do not clump, making it difficult to smooth into powder. The yield that gets higher as the speed of agitation increases is also related to 12 the glass transition temperature of the sugar. Due to the use of sugar as a filler, the faster the agitation speed will support the more optimal heat spread and will continuously accelerate the change in phase or form of sugar. Therefore, more products are produced and not wasted because they burn because they stick to the pan and do not get intense contact at high temperatures. It can be proven through observations that the lower the agitation speed, the more the remaining processing results are attached to the crystallizer pan.

3.2.Moisture Contents and Ash Contents

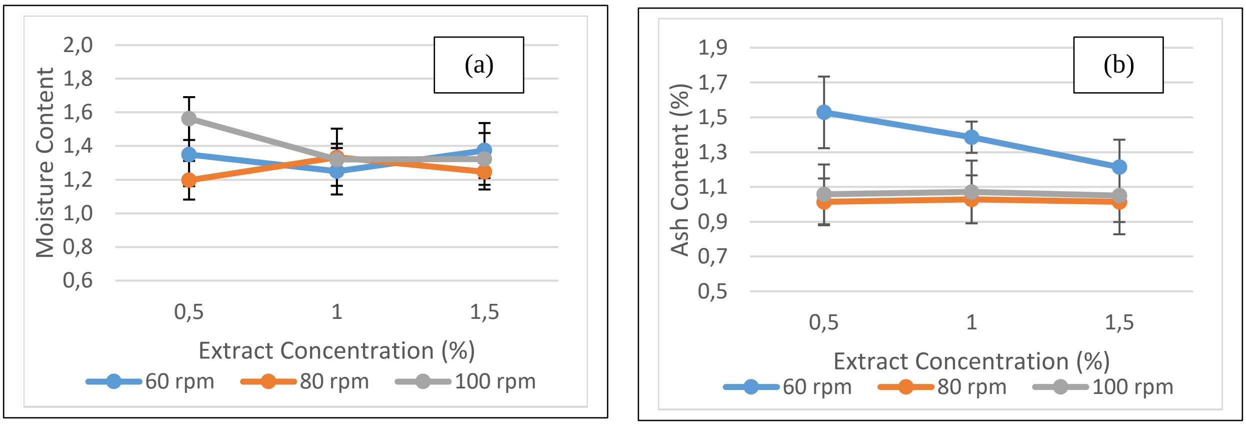

Figure 2 (a) Moisture Contents (b) Ash Contents

Based on the Indonesian National Standard (SNI) of traditional powder drinks (SNI. 01-4320-1996), the maximum moisture content in traditional powdered beverage products is as much as 3%. Water content has a relationship with storage factors and moisture of water vapor (Arizka & Daryatmo, 2015). The moisture content of the powdered drink sample in all treatments is in accordance with the standards of the SNI, where there is no value above 3%. Water content of all samples in the range of 1.20 to 1.56 (Figure 2).

The speed of agitation gives a significant difference ($p<0.05$) on the yield of the percentage of ash content of the instant ginger powder drink product. Changes in the speed of agitation in crystallizers have an indirect influence on the percentage of ash content from samples of instant ginger powder drinks. Intensive stirring at the appropriate speed when the crystallization process begins to occur will cause heat can be evenly dispersed (Mursalin et al., 2019). Materials that absorb heat more evenly will produce products with a lower ash content. As for the treatment of the addition of *Andrographis paniculata* extract, it did not have a noticeable influence / difference ($p>0.05$) on the ash content value of instant ginger powder drinks.

3.3.Crystallization Time and Dissolving Time

The downward trend in crystallization time can be seen in Table 1. In this case, the crystallization time follows the theory that the right stirring speed can produce homogenous crystallized powder and accelerate the crystal formation rate (Mullin, 2001). There was a significant decrease in the downward trend when the crystallizer agitation speed increased from 60 rpm to 80 rpm. Meanwhile, when the speed of the crystallizer agitation increased from 80 rpm to 100 rpm, the decrease was not too significant. In general, the crystallization time at 100 rpm treatment is the shortest crystallization process. Based on the data from the measurement of the crystallization time of the powder drink production process at each treatment, the fastest time to form crystals was obtained at an agitation speed treatment of 100 rpm, with an average time of 109 minutes. In this case, the time for one production process with much time can be considered quite long. It can occur due to starch from ginger rhizomes that have been gelatinized during the boiling process in manufacturing extract. Starch content in rhizome-type plants, such as ginger, can cause the crystallization process in sugar to last longer. This starch also causes gelatinization, which can increase the viscosity of the solution during the heating process (Desnita et al., 2019). Heating causes the kinetic energy between water molecules to be stronger than the attraction between starch molecules, which causes water to enter the starch and expand (Krisna, 2011). The increase in viscosity and the expansion of starch results in the time it takes for the breakup of the dough into crystalline powder to increase. Therefore, the manufacture of instant powdered beverages requires the decantation or deposition of starch before the heating process (Desnita et al., 2019). Decantation is necessary to do before boiling the extract.

Table 1. Crystallization Time (minutes)

Agitation speed (rpm)	*A paniculate* extract		
	0,5%	1%	1.5%
60	129.00±9.20^{a,NS}	128.00±9.19 ^{a,NS}	126.00±9.19 ^{a,NS}
80	111.00±2.83^{b,NS}	111.00±2.12 ^{b,NS}	111.00±4.95 ^{b,NS}
100	109.00±7.78^{b,NS}	108.00±7.78 ^{b,NS}	110.00±2.12 ^{b,NS}

Based on the results of the two-way ANOVA statistical test, the change in the speed of agitation gave a noticeable difference ($p<0.05$) at the soluble time of the instant ginger powder drink>. The decrease in soluble time is directly proportional to the increase in the agitation speed of the crystallizer machine (Table 2). The higher the speed of agitation used, the shorter the soluble time of the resulting powder. This relates to the flatness or uniformity of the associated sample. The presence of a constant and intensifying stirring speed will spread heat evenly in the processed sample (Mursalin et al., 2019). Intensified speed will increase uniformity for moisture content parameters of the product itself.

Table 6. Dissolving Time (second)

Agitation speed (rpm)	A paniculate extract concentration		
	0,5%	1%	1.5%
60	107.00± 3.83^{c,NS}	104.00±3.25^{c,NS}	100.00±2.35^{c,NS}
80	84.00±4.22^{b,NS}	87.00±3.93^{b,NS}	84.00±3.31^{b,NS}
100	70.00±3.67^{a,NS}	71.00±2.79^{a,NS}	70.00±2.58^{a,NS}

One of the factors that can affect the soluble time of the product is the moisture content of the related product, where the lower the moisture content of the instant powdered drink, the shorter the soluble time needed for the product to dissolve (Permata & Sayuti, 2016). An increase in the moisture content in food products leads to the formation of bonds that causes the formation of clots that will increase the duration of time it takes to break up the bonds between those particles. Instant ginger powder drink is a food product that is hygroscopic. The moisture content in a material that is hygroscopic is water that is fixedly bound in the material because it is covered by the presence of capillaries. Permata & Sayuti (2016) states that the water content is high on the material may lower the degree of solubility of the product. The presence of water will interfere with the course of the reconstitution process, which will result in clumping at the time of adding water before consumption. This can affect the evenness or uniformity for the moisture content parameters of the product resulting in no correlation between the percentage value of the moisture content and the soluble time of the product.

The use of sugar as an encapsulant or filler is also one of the factors that affect the soluble time of instant ginger powder drink products. Haryanto (2018) stated that sucrose has a high solubility to water. If the concentration of sucrose is higher, the solubility of powdered beverage products in water will be higher. In this study, the same sugar treatment was used in each sample, so that it did not have a significant effect on the soluble time of the instant ginger powder drink product.

3.4.Acidity Level (pH Value)

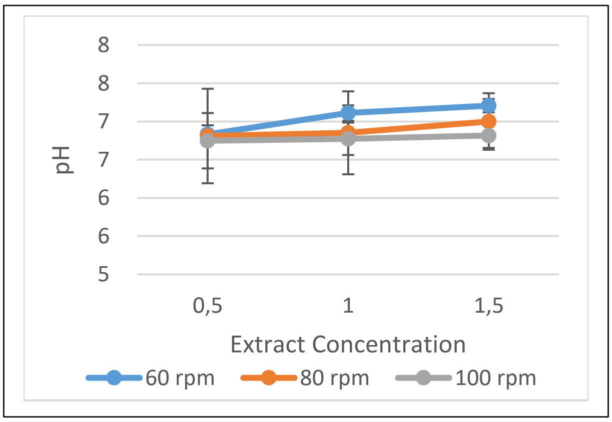

Figure 4. Acidity Level (pH Values)

The acidity level of the material used is something that needs to be considered before making instant powder drinks by the crystallization method. The material should not have too low a pH, because in conditions of low pH there will be a reaction of inversion of sucrose into fructose and glucose which can cause caramelization (Desnita et al., 2019). Before the pH measurement of instant ginger powder beverage products, pH measurements are carried out on the *Andrographis paniculata* extract used. From the pH measurement of *Andrographis paniculata extract* that has been carried out, the pH data of Andrographis paniculata extract was obtained by 9.87.

Based on the research data that has been obtained, it can be seen that the trend of increasing the pH value is directly proportional to the increase in the concentration value of the addition of *Andrographis paniculata* extracts. Where in samples with the treatment of adding *Andrographis paniculata* extract 0.5% the average pH of the product obtained was 6.79 which then increased to 6.91 at a concentration of 1.0% and 7.0 at a concentration of 1.5%. An increase in the concentration of the extract may increase the pH value of the final product of the powdered drink. Nevertheless, this increase is not very significant due to the difference in the addition of extracts between concentrations that are not too far away. The pH value of the instant ginger powder drink product in this study was in the range of pH 6-7.

The pH value of the material used can affect the crystallization rate. The rate of caramelization can be accelerated under acidic conditions. At a low pH, the movement of the reactant molecule will be stronger which will increase the reaction rate (Desnita et al., 2019). The difference between concentrations that are not too far does not have a significant influence on the crystallization rate because the pH range is too tight.

3.5.Antioxidant activity

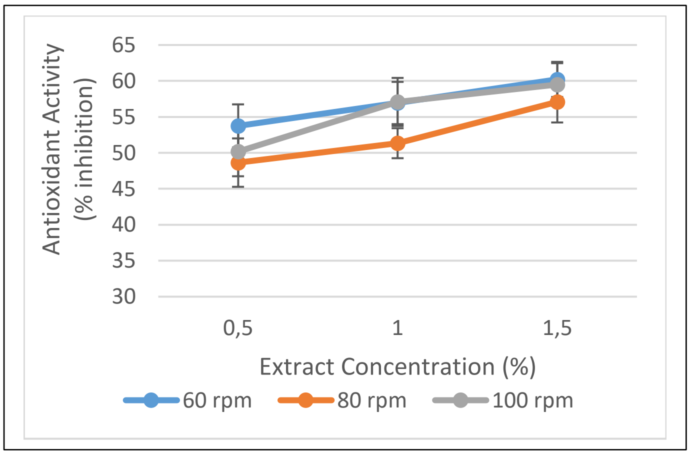

Figure 5. Antioxidant Activity

Antioxidant compounds contained in ginger are gingerol and shogaol (Embuscado, 2015). The gingerol content in oleoresins ranges from 14%-25%, while shogaol is 2.8%-7.0%

(Srinivasan, 2017). Gingerol compounds have anticoagulant properties, where these compounds can prevent blood clots and improve blood circulation so that they can prevent the onset of heart disease, stroke and various degenerative diseases (Stoilova et al., 2007). *Andrographis paniculata* is one of the herbal plants known to contain antioxidant compounds. Antioxidant compounds contained in the plant are andrographolide compounds and flavonoids. Andrographolide is a colourless compound with anti-HIV, anti-inflammatory and antineoplastic properties commonly found in leaves with a concentration of 0.054 - 4.686% (Sharma et al., 2018). Andrographolide compounds provide bitter characteristics to *Andrographis paniculata* (Thakur et al., 2015). They have pharmacological effects as antivirals against several viruses such as influenza A, Hepatitis B, Hepatitis C, Human Papilloma and Epstein-Barr (Gupta et al., 2017). Andrographolide compounds can significantly fight COVID-19 by inhibiting the main protease enzyme SARS CoV-2 (Rajagopal et al., 2020).

Based on antioxidant tests that have been carried out on each ingredient, antioxidant activity levels from ginger rhizome extract, with an average of 17.77% and Andrographolida extract, with an average antioxidant activity of 63.32%, were obtained. Based on the statistical test, changes in the concentration of Andrographolida extract addition have a significant effect ($p<0.05$) on the percentage value of the antioxidant activity of the instant powder drink. The mixing of spices in beverage formulations can be done to obtain a combination of antioxidants with higher activity than if only used separately.

The antioxidant activity of instant powder drinks has an average antioxidant activity of more than 50%. Based on the data, the increase in the agitation speed affects the decrease in the antioxidant activity of the product. The graph of the results of the activity analysis shows a downward trend toward the value of antioxidant activity as the speed of crystallizer agitation increases. Accelerating agitation speed causing the material to react faster due to contact with an increasingly intense heat source. Antioxidant compounds include easily damaged when exposed to oxygen, light, high temperatures and drying. Ramdhani et al. (2013) stated that the higher the processing temperature of a product, the lower the antioxidant activity. Antioxidant activity resulting from instant ginger powder drink products Andrografolid compounds provide bitter characteristics to Andrografolid (Thakur et al., 2015) and have pharmacological effects as antivirals against several groups of viruses such as influenza A, Hepatitis B, Hepatitis C, Human Papilloma and Epstein-Barr (Gupta et al., 2017). Andrographolide compounds can significantly fight COVID-19 by inhibiting the main protease enzyme SARS CoV-2 (Rajagopal et al., 2020). this time it was quite high, and there was no significant decrease in antioxidant activity compared to the antioxidant activity of the ingredients used. It is due to the use of sugar as a filler that can be used to re-crystallize a food ingredient and provide food stability and a better taste (Gaman & Sherrington, 1994 in Haryanto, 2018).

Granulated sugar as a filler provides a sweet taste, accelerates the crystallization process, and is a preservative in instant ginger products (Gafar et al., 2018). Granulated sugar acts as an encapsulant agent in the crystallization process. Idham et al. (2012) mentioned that encapsulation techniques reduce the interaction of foodstuffs with environmental factors, such as temperature, light, humidity, and oxygen. It leads to the content of Antioxidant compounds in the product can be protected during the crystallization process by high-temperature heating so that the desired percentage of antioxidant activity can still be achieved.

4. Conclusion

The agitation speed treatment has a significant effect on reducing the crystallization time and dissolving time of instant ginger powder drinks. The crystallization treatment with an agitation speed of 100 rpm significantly reduced the crystallization time. The agitation speed treatment also significantly increases the percentage of the crystallization yield. Crystallization with an agitation speed of 100 rpm gives the largest yield and the most homogeneous particle size.

The treatment of adding concentrations of *Andrographis paniculata* extract has a significant effect ($p<0.05$) on the antioxidant activity of instant ginger powder drinks, where the addition of *Andrographis paniculata* extract with a concentration of 1.5% provides the highest antioxidant activity. The best combination of treatments that produce products with the best physical and chemical characteristics is the crystallization carried out with the agitation speed is 100 rpm and with the addition of *Andrographis paniculata* extract 1.5%.

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