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Evaluation Of The Effect Of Particle Size Distribution Of Quail Egg (Coturnix Coturnix) On Acid Neutralization Capacity And Velocity

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Abstract:
Background*: The acid neutralizing capacity of antacids is the ability of tablets or liquid formulations of antacids to neutralize stomach acid at $37^0C \pm 2^0C$ and is expressed in milliequivalents. One of the uses of natural materials as acid neutralization is quail egg shells because they contain very high $CaCO_3$. The use of calcium carbonate in the pharmaceutical field is as an antacid because of its ability to neutralize acids.*
Materials and Methods*: Quail eggshell powder was purified from its organic compound content, then quail eggshell powder was characterized including identification using Fourier Transform Infra-Red (FTIR) and crystallinity using X-Ray Diffraction (XRD). Particle size, $CaCO_3$ content, capacity and rate of acid neutralization of eggshell powder were evaluated for each powder size range in 20 – 40 mesh (sample 1); 40 – 60 mesh (sample 2); 60 – 80 mesh (sample 3); 80 – 100 mesh (sample 4); ≥100 mesh sieve (sample 5).*
Results*: The study revealed that the FTIR spectrum of quail eggshell powder was 96% similar to that of $CaCO_3$. The acid neutralizing capacities of samples 1, 2, 3, 4, 5 were 21.82; 22.17; 22.61; 23.0; 24.03 mEq and the speed of acid neutralization in each sample sequentially at 23, 22, 20, 18, 17 minutes.*
Conclusion: *Variations in particle size distribution have a significant effect on the value of the capacity and speed of acid neutralization, in which the smaller the particle size, the higher the value of the capacity and speed of acid neutralization*
Key Word*: Quail Egg Shell, Acid Neutralization Capacity, Acid Neutralization Speed, PSA, XRD, FTIR*

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I. Introduction

Acid neutralizing capacity (ANC) is the ability of a material to neutralize acid and is expressed in milliequivalents (mEq). Drug dosage form that contain ingredients to neutralize stomach acid are called antacids. One of the good properties of antacids is determined by the high value of acid neutralizing capacity [1]. According to the Food and Drug Administration, the acid neutralizing capacity of antacids is ≥ 5 mEq. The greater the acid neutralizing capacity value, the higher the effectiveness of antacids (Rao dkk., 2018). The acid neutralizing capacity of antacids is the ability of tablets or liquid formulations of antacids to neutralize stomach acid at $37^0C \pm 2^0C$ and is expressed in milliequivalents [2]. The neutralizing capacity of this acid is affected by the type of active substance, crystal form, suspension, water content, and type of disintegrant. Based on their chemical content and structure, antacids are grouped into carbonate and hydrogen carbonate groups such as $CaCO_3$, $MgCO_3$, $NaHCO_3$, hydroxyl groups such as $Mg(OH)_2$ and $Al(OH)_3$, and compound groups that have a layered structure such as hydrotalcite and magaldrate [1].

One of the uses of natural materials as acid neutralization is quail egg shells because they contain very high $CaCO_3$. Based on statistical data on livestock and animal health, it shows that the production of quail eggs in 2013-2017 in Indonesia has continued to increase, in 2013 the production of quail eggs was 18,936 tons, in 2014 there were 20,709 tons, in 2015 as many as 22,131 tons, in 2016 as many as 23,575 tons, and in 2017 as many as 25,272 tons. The increasing number of quail egg production in Indonesia is due to the increasing consumer demand for consuming quail eggs, so that many quail egg shells become waste that is no longer used and just thrown away. The use of waste continues to be carried out by researchers. Utilization of this waste aims to get products that are more useful, products that can be renewed, products that can increase economic selling value and can be utilized by humans. In addition, the utilization of waste can also reduce environmental pollution. One of the many wastes found in Indonesia is egg shells [3]. Egg shells can be used as a natural acid neutralizing agent or as an antacid. According to Butcher and Miles (1990) the eggshell component consists of 97% calcium carbonate ($CaCO_3$).

The use of calcium carbonate in the pharmaceutical field is as an antacid because of its ability to neutralize acids [4]. The composition of egg shells generally consists of water (1.6%) and dry matter (98.4%). Of the total dry matter in the egg shell, it contains mineral elements (95.1%) and protein (3.3%). Based on the existing mineral composition, the eggshell is composed of $CaCO_3$ crystals (98.43%); $MgCO_3$(0.84%) and $Ca_3(PO_4)_2$ (0.75%) [5]. According to Yonata et al's research, (2017) the value of calcium levels in quail egg shell flour was 9.46% higher than the calcium levels in purebred chicken egg shells 6.41% and free-range chicken 5.22% [6]. The use of eggshells has also been studied by Izzaturrohmah et al, (2016) by making Fast Dissolve Tablets (FDT) preparations from eggshells and obtained effective results to quickly neutralize acid but this effectiveness has not been affected by the size distribution of eggshell particles [7]. According to Rao et al, (2018) the size of the particle distribution can affect the effectiveness of antacids. This is supported by Alghofar's research data, (2017) regarding the analysis of the acid neutralizing capacity of various antacid dosage forms, the value obtained was > 5 mEq with the order of the highest ANC value, namely suspension preparation 10.6 tablets 10.5 and powder 9.6 so that meet the requirements of the acid neutralization test [8,9]. Compared to the tablet form, liquid antacid formulations are generally preferred because they have a higher neutralizing capacity, which is due to the smaller particle size distribution and larger surface area.

Determination of the acid neutralizing capacity was also carried out on plants, based on Dewi's research, (2012) regarding the acid neutralizing capacity of the in-vitro rhizome of temu putih which resulted in a pH value that was still in the acidic category so that the test results showed that the acid neutralizing capacity test could not be carried out on infusions Curcuma rhizome as an antacid [10]. Apart from Dewi, researchers Houshia et al. (2013) also investigated the acid neutralizing capacity of ginger, potato, cucumber, almond and turmeric plants [11]. The results obtained show that the composition of each of these plants can fight acid (antacids). So from these studies the natural ingredients used have not been able to neutralize the acid. In addition to the acid neutralizing capacity, to see the effect of particle size distribution on acid neutralization, the speed of acid neutralization is also carried out, where the faster the acid neutralizes, the better the effectiveness of antacids. Based on the above background, researchers are interested in utilizing natural materials from quail egg shells containing calcium carbonate ($CaCO_3$) as acid neutralizers with variants of particle size distribution, which aims to determine the effect of particle size distribution on the capacity and speed of acid neutralization. (10)

II. Material And Methods

Tools and materials

The tools used in this study were stampers and mortars, sieves with nos 20, 40, 60, 80, 100, Particle Size Analyzer (PSA), X-Ray Diffraction (XRD), Fourier Transform Infra-Red (FTIR), burettes and stative, oven, analytical balance, magnetic stirrer, and pH meter. The materials used in this study were quail egg shells, Calcium carbonate (Merck), aquadest, HCl (Merck), NaOH (Merck), hydroxy naphthol blue P, Na_2EDTA (Merck), $ZnSO_4$ (Merck), Erichrom Blact T (Merck), Sodium Tetraborate/Borac (Merck), Methyl Orange (Merck), Oxalic Acid (Merck), and Phenolphthalein/PP (Merck).

Procedure methodology

Cleaning and Separation of Organic Compounds from Egg Shells

Before processing, the egg shells are cleaned of mucus and then mechanically separated from the membrane. The egg shells were washed under running water and sterilized by autoclaving (121^{o}C, 15 minutes) then dried in an oven (40^{o}C, 24 hours). The eggshells are pounded in a mortar and then sieved (mesh 10) to separate the membrane from the eggshell (the membrane will remain in the sieve, while the eggshell will pass through the sieve).

Grinding and purification of quail egg shell powder

Grinding of quail eggshell powder was done manually using a stamper and mortar. Purification of quail egg shell powder was carried out by boiling it in water (3 L) for one hour. Substances that float to the top when boiling are removed. The precipitate of egg shell powder was dried in an oven (160^{o}C, 2 hours).

Separation of Powder Based on Size Range

The distribution of quail eggshell powder using a sieve from mesh 20 to mesh 100, so that what is left in 20-40 mesh is called sample 1, what is left in 40-60 mesh is called sample 2, what is left in 60-80 mesh is called sample 3, what is left in 80-100 mesh is called sample 4, and more than 100 mesh is called sample 5.

Physicochemical Characterization of Eggshell Powder

a. Particle size

The sample particle size in each mesh sieve fraction 20, 40, 60, 80, and 100 was measured using a laser diffraction particle size analyzer (Beckman Coulter® type LS I3 320)

b. Crystallinity Test

The purified powder was analyzed for crystallinity using an X-Ray Diffraction (XRD) instrument and then compared with standard calcium carbonate from ICSD (Inorganic Structure Crystal Database).

c. Fourier Transform Infrared (FTIR)

The functional groups of the samples were analyzed and the percentage similarity to standard calcium carbonate was seen using FTIR spectral images (Agilent Technologies Carry 630®).

Determination of Calcium Carbonate ($CaCO_3$) Levels

Determination of calcium carbonate ($CaCO_3$) content from egg shell powder was determined by the volumetric method. Each of samples 1, 2, 3, 4 and 5 was carefully weighed approximately 200 mg of the substance which had been dried at 200°C for 4 hours. Put it in a 250 mL beaker, moisten with a few ml of water, add enough 3 N hydrochloric acid drop by drop until completely dissolved. Add 100 mL of water, 15 mL of sodium hydroxide 1 N and 300 mg of hydroxynaphthol P blue. Titrate with 0.05 M LV of edetate disodium which has previously been standardized with $ZnSO_4$, titrate with edetate disodium until the blue end point. Each mL of 0.05 M edetate disodium is equivalent to 5.004 mg $CaCO_3$ [12].

Determination of Acid Neutralizing Capacity

The method for determining the neutralizing capacity is based on the Indonesian Pharmacopoeia V edition, namely: Test solution, the powder is weighed carefully for the amount of test substance as stated (1.25 gram) in each monograph, put into a 250 mL beaker, add 70 mL of water and mix using magnetic stirrer for 1 minute.

[note: that all tests were carried out at 37°C ±3°].

a. Standardization of pH meters

The pH meter was calibrated using a standard buffer solution of potassium biphthalate 0.05 M (dissolve 10.12 g $KHC_8H_4O_4$ which has been dried at 110° for 1 hour in water up to 1000 mL) and potassium tetraoxalate 0.05 M (dissolve 12.61 g $KH_3(C_2O_4)_2\ 2H_2O$) as stated in the determination of pH [12].

b. Magnetic stirrer

100 mL of water is placed in a 250 mL beaker containing a 40 mm x 10 mm magnetic stir bar coated with solid perfluorocarbons and having a rotating ring at the center. the power of the magnetic stirrer is adjusted to produce a mean stirring speed of 300±30 revolutions per minute, when the stirring rod is centered in the beaker, as determined by a suitable optical tachometer.

c. Determination of acid neutralizing capacity

30.0 mL of hydrochloric acid 1.0 N LV was added to the test solution. [note if the acid neutralizing capacity of the test substance is greater than 25 mEq, use 60.0 mL of hydrochloric acid 1.0 N LV]. After addition of acid, stir for 15 minutes, immediately titrate immediately. Titrate the excess hydrochloric acid with sodium hydroxide 0.5 N LV in no more than 5 minutes until a stable pH of 3.5 is reached (10 seconds to 15 seconds). Calculate the amount of mEq of the acid used with the formula:

Total mEq = $(30 \times N_{HCl}) - (V_{NaOH} \times N_{NaOH})$

N_{HCl} and N_{NaOH} are the normality of LV hydrochloric acid and LV sodium hydroxide, respectively, V_{NaOH} is the volume of LV sodium hydroxide used for titration. Results are expressed in mEq of acid used per g of test substance [12].

d. Determination of Acid Neutralization Speed

This determination was carried out by adding 70 mL of distilled water to 1.25 grams of sample in a 250 mL beaker, then adding 1.25 mL of HCl 1 N, stirring using a magnetic stirrer, the temperature was maintained at 37°C and stirring at a speed of 200 rpm. Check the initial pH before adding HCl 1 N then check the change in pH after adding HCl 1 N every minute until the pH is stable and does not change.

III. Result

Table no 1: Results of Particle Size Analysis

Sample	Average Particle Size (µm)
Sample 1 (20-40 Mesh)	-
Sample 2 (40-60 Mesh)	812.5 ± 317.8
Sample 3 (60-80 Mesh)	484.2 ± 163.8
Sample 4 (80-100 Mesh)	330.3 ± 88.90

Sample 5 (≥100 Mesh)	254.0 ± 82.97

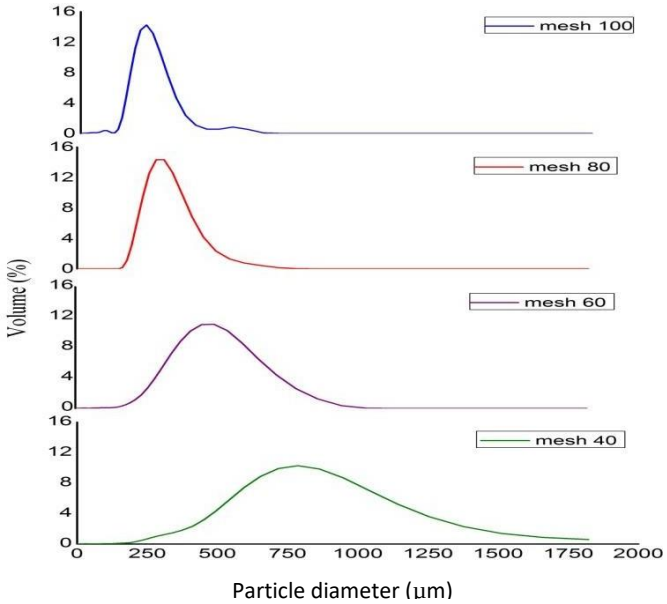


Figure 1: PSA graph of quail egg shell samples

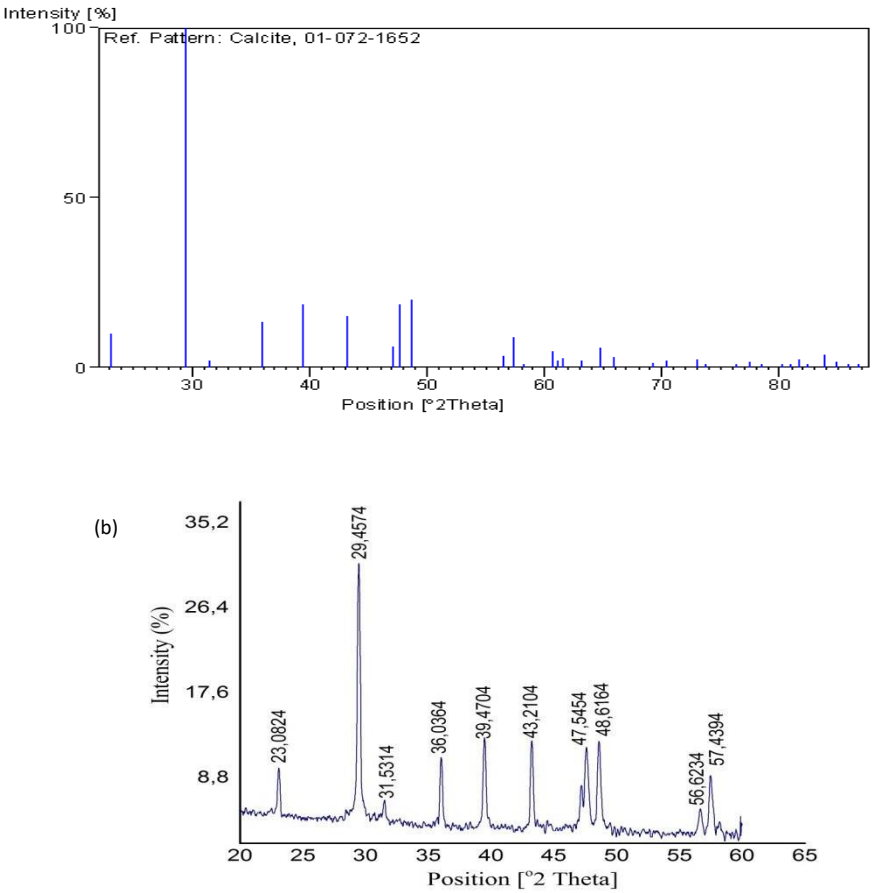


Figure 2: XRD diffractogram of (a) standard calcium carbonate; (b) quail egg shell powder

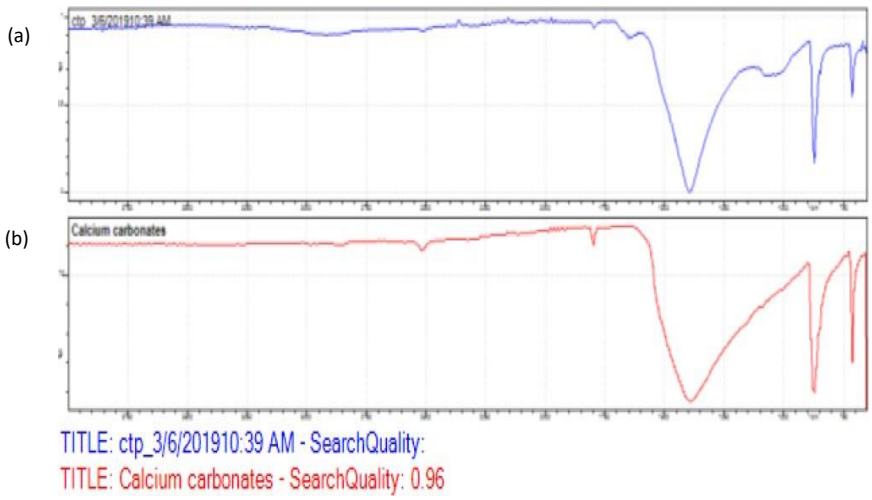


Figure 3: FTIR spectrum (a) quail egg shell powder; (b) standard calcium carbonate

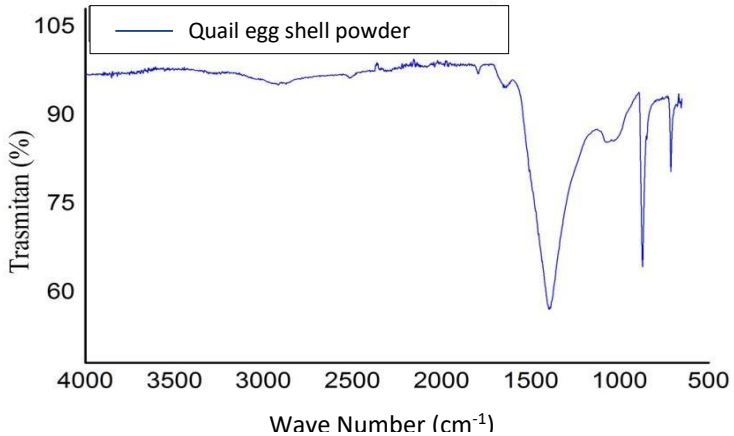
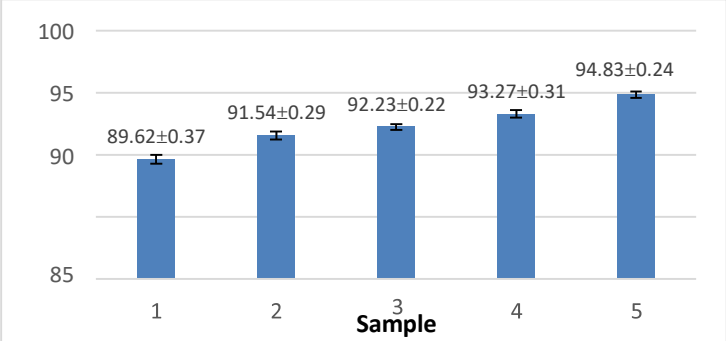


Figure 4: Quail egg shell spectrum

Table no 2: Standardization of Na_2EDTA Titration

No.	$ZnSO_4$ weight (mg)	Titer Volume (mL)	Molarity of Na_2EDTA (M)	Average Molarity of Na_2EDTA (M)
1	50.1	3.5	0.0498	0.05
2	50.0	3.4	0.0511	
3	50.0	3.5	0.0497	



Gambar 5: Determination of $CaCO_3$ levels in quail egg shells

Table no 3: Results of Standardization of NaOH with Oxalic Acid

No.	Oxalic Acid Volume (mL)	Concentration of Oxalic Acid (N)	NaOH Volume (mL)	Concentration of NaOH (N)	Average Normality of NaOH (N)
1	10	0.5	10	0.5	0.4983
2	10		10	0.5	
3	10		10.1	0.495	

Table no 4: Result of standardization of HCl with sodium tetraborate (borac)

No.	Borac Volume (mL)	Concentration of Borac (N)	HCl Volume (mL)	Concentration of HCl (N)	Average Normality of HCl (N)
1	25	0.1	2.6	0.96	0.9866
2	25		2.5	1	
3	25		2.5	1	

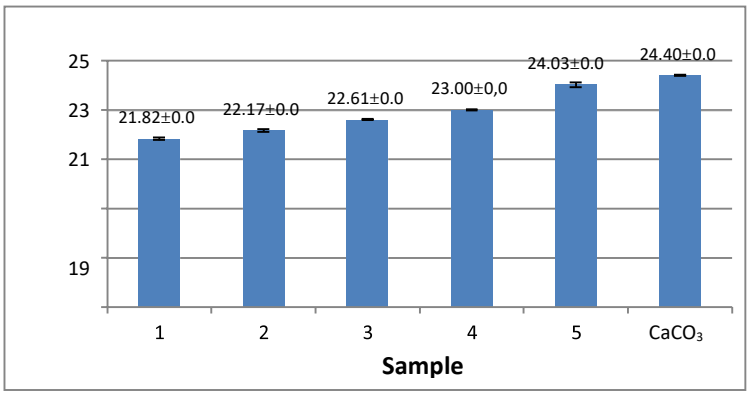


Figure 6: Acid Neutralizing Capacity Value

Table no 5: Result of Acid Neutralization Rate

Sample	Times (Minutes)	pH
Standard (CCC)	15	7,74 ± 0,02
Quail Egg Shell (First Sample)	17	7,74 ± 0,02
Quail Egg Shell (Second Sample)	18	7,7 ± 0,01
Quail Egg Shell (Third Sample)	20	7,65 ± 0,01
Quail Egg Shell (Fourth Sample)	22	7,5 ± 0,02
Cangkang Telur Puyuh (Fifth Sample)	23	7,46 ± 0,01

IV. Discussion

In this study the material used was quail egg shells. Quail eggs were obtained from Batununggal, Bandung, as much as 8 kg. Quail eggshell powder production is done by grinding manually using a mortar and stamper. The egg shells were first washed using running water then dried using an oven at 40ºC for 24 hours to reduce the water content, then sterilized using an autoclave at 121ºC for 15 minutes then dried again using an oven (40ºC, 24 hours). The purpose of the sterilization process is to kill all microorganisms, both pathogenic and non-pathogenic, from the shells of quail eggs. The sterilized shells were weighed and the result was 678 grams so that it could be expressed as almost 9% by weight of the eggshells of the total weight of the whole quail egg. The eggshells are pounded using a mortar and stramper and then sifted (mesh 10) to separate the membrane from the eggshell (the membrane will be retained in the filter, while the eggshell will pass through the filter). Weigh the results of the sifted shells (mesh 10) and the results are 620 grams.

Purification of egg shell powder from organic compounds by boiling 620 grams of powder in 3.72 L of distilled water for one hour. Substances floating on top during boiling are discarded. The precipitated eggshell powder was sterilized again using an oven (160ºC for 2 hours). Separation of eggshell powder based on size range using sieve with mesh no. 20, 40, 60, 80, and 100. Shell powder retained on sieve no. 20-40 mesh is called sample 1, retained on sieve no. 40-60 mesh is called sample 2, retained on sieve no. 60-80 mesh is called sample 3, retained on sieve no. 80-100 is called sample 4, and those retained on sieve no. more than 100 mesh are called sample 5.

In this research, three quail eggshell characterizations were carried out, namely Particle Size, Crystanillity Test, and Fourier Transform Infra-Red (FTIR).

a. Particle Size

Particle size was measured using the Particle Size Analyzer (PSA) instrument. Particle size can be known through the resulting images [13]. PSA analysis was carried out with the aim of knowing the quantitative size of the calcium carbonate particles in quail egg shells based on a sieve of 20 to 100 mesh. The results of the sample PSA analysis are shown in Table I and Figure 1.

Based on the figure 1, the smaller the mesh size, the graph shows the smaller particle diameter, while the larger the mesh size, the larger the diameter. The PSA test was not carried out on mesh 20 because its size exceeded the minimum limit of the PSA test.

b. Cristanillity Test

Testing the crystallinity of a compound is measured using X-ray diffraction. Testing using X-ray diffraction is a common technique used to determine the crystallographic characteristics of a material through the intensity peaks that appear [14]. Compounds from quail eggshell powder samples were analyzed by XRD (X-Ray Diffraction). This analysis aims to look at the diffractogram peaks in quail egg shell powder samples and then compare them with the character peaks of calcium carbonate ($CaCO_3$) from ICSD data using POWD-12++ [15]. In the following, the X axis represents the angle 2Ɵ and the Y axis represents the x-ray intensity after passing through a sample of quail eggshell powder. In the quail egg shell powder sample test using an angle of 2Ɵ which is used is between 5-60º and peaks appear between angles of 23-60º. XRD results can be seen in Figure 2.

Based on the results of the diffractogram of the sample on the figure 2, there are ten highest peaks in the sample as markers of the compound and have a peak angle of 2Ɵ corresponding to calcium carbonate. namely 23.09; 29.45; 31.49; 36.00; 39.46; 43.23; 47.57; 48.57; 56.65; 57.46º and matched with standard calcium carbonate ($CaCO_3$) data from ICSD using POWD-12++ with a peak angle value of 2Ɵ, namely 23.06; 29.45; 31.54; 35.96; 39.42; 43.16; 47.64; 48.58; 56.56; 57,49). These results indicate that the diffraction results of quail egg shell powder produce a pattern of peaks that corresponds to the peaks of $CaCO_3$.

c. Fourier Transform Infra-Red (FTIR)

FTIR analysis aims to identify $CaCO_3$ compounds from quail egg shell samples. Comparison of the results of sample characterization with standard $CaCO_3$ can be seen in Figure 3 while the data on sample characteristics using FTIR can be seen in Figure 4.

Figure 4 shows that quail eggshell powder was measured at wave numbers 650 – 4000 cm^{-1} with a transmitter percentage of 62% -101%. Peaks at wave numbers 633 and 3570 cm^{-1} indicate vibrations from OH, 3400 cm^{-1} indicate OH stretching vibrations, carbonate bonds observed at 870 and 1430 cm^{-1}, 1400 cm^{-1} indicate CO_3 asymmetric stretching vibrations, 800 cm^{-1} indicate deformation outside the CO_3 field [16]. When compared with the standard $CaCO_3$ spectrum, there are similarities in the shape of the spectrum which indicates the similarity of functional groups and standard $CaCO_3$ compounds with quail egg shell powder. The equation is 96%. This shows that quail egg shells contain $CaCO_3$.

d. Determination of CaCO3 Levels from Quail Egg Shell Powder

Determination of calcium carbonate levels in quail egg shells was carried out by complexometric titration using a Na_2EDTA titrant because calcium ions can form complexes with EDTA. EDTA is a compound that is easily damaged so that EDTA is standardized first to get the actual concentration of EDTA. Standardization of EDTA is standardized with $MgSO_4$ and uses the Erichrom Black T (EBT) indicator. Erichrom Black T has a pH of 7-11 and the color change that occurs is from purplish red to blue because during the titration H+ ions are released, the solution to be titrated is previously added with buffer with a pH of 10. The purpose of adding this buffer is to maintain the pH value so that when adding acid, base or water did not change the pH significantly (Warsy et al, 2015). When an indicator is added before the titration, the indicator will form a complex with Mg^{2+} (red in color) then Mg^{2+} in the complex will react with EDTA, the red color will disappear, then a little excess of EDTA causes an end point, namely the occurrence of a blue color and the reaction that occurs is as follows [17].

$Mg\ Ind^-_{(aq)} + H2Y^{2-}_{(aq)} \rightarrow MgY^{2-}_{(aq)} + H\ Ind^-_{(aq)} + H^+_{(aq)}$

(red) (colorless) (blue)

The results obtained for the volume of Na_2EDTA can be seen in Table II. So that the results obtained can be calculated the actual concentration of Na_2EDTA using the resulting dilution formula of 0.05 M according to the desired Na_2EDTA solution.

After obtaining the actual value of Na_2EDTA concentration, then proceed with titration to determine the level of $CaCO_3$ in quail egg shells. First, each quail eggshell sample was dried in an oven at 200°C for 4 hours. This is so that the sample to be studied is not contaminated with unwanted substances. The sample is added with distilled water and HCl 3 N. The purpose of dissolving in HCl is for the hydrolysis process and will form gas bubbles in the solution so that the sample dissolves completely. In the next stage, distilled water, NaOH 1 N and hydroxynaphtol blue indicator were added. The addition of NaOH aims to neutralize and alkalinize the solution. After that it was titrated with Na_2EDTA, the color change that occurred was from purplish red to dark blue. This treatment was carried out in triplicate for each sample to obtain accurate results. From the results of the volume of Na_2EDTA produced, the $CaCO_3$ levels in the quail egg shells can be calculated where the results obtained can be seen in Figure 5.

Based on the results of these data, the highest $CaCO_3$ level was found in sample 5 (≥100 mesh), namely 94.83% and according to research by Warsy et al (2016) the $CaCO_3$ level in chicken egg shells was 92.57%, this indicates that the $CaCO_3$ level in the eggshell Quail is higher than chicken egg shells. Based on the particle size, the smaller the particle size, the higher the $CaCO_3$ content in the eggshell. This is because in samples that have a larger particle size, it is possible that there is still egg membrane in it so that the level value is lower, while for samples with smaller particle sizes, a little egg membrane is left behind and the $CaCO_3$ contained in it is more so that the level value in the sample 5 (≥100 mesh) is the highest content value in quail egg shells. The size of the particle distribution can affect the value of the $CaCO_3$ content in the quail eggshell [18]. To ensure a significant difference between each sample, an analysis was carried out using SPSS One Way Anova and Post Hoc Test LSD.

In the ANOVA test the data must be evenly distributed with a significant value (P> 0.05). The data used in ANOVA has a significant value of 0.908, meaning that the value is more than 0.05. This shows that the data is evenly distributed so that the One Way Anova test can be carried out. The results of the ANOVA test using the F test, obtained a calculated F value of 131,037 with a significant value of 0.00. Decision making is based on a comparison of F_{count} and F_{table}, if F_{count} is smaller than Ftable ($F_{count}<F_{table}$) then H0 is accepted. From the ANOVA results, the calculated F value is greater than F_{table} (3.48), so H_1 is accepted and H_0 is rejected. This shows the influence of the size distribution of quail egg shell particles on the determination of $CaCO_3$ levels. The next test uses LSD, where the LSD test aims to see significant differences between each sample. LSD test results show a significant or significant difference if the value is significant (<0.05). Based on the data, each sample has a significant difference, sample 1 (20-40 mesh) has a difference with samples 2 (40-60 mesh), 3 (60-80 mesh), 4 (80-100 mesh), and 5 (≥100 mesh) as well as other samples all have significant difference. This shows that there is an effect of the size distribution of quail egg shell particles on the determination of $CaCO_3$ levels because based on the LSD test all data from each sample has a significant difference.

The acid neutralizing capacity is defined as the milliequivalent (mEq) value of HCl 1N with a stable pH of 3.5 [19]. According to the Food and Drug Administration, the acid neutralizing capacity of antacids is ≥ 5 mEq per dose. The greater the value of the acid-neutralizing capacity, the higher the effectiveness of the antacids [8]. To determine the ANC value, powder samples from quail egg shells were used which had a particle size distribution ranging from 20 to 100 mesh. In the initial stage, 1,250 mg of sample was weighed. The number of samples used was in accordance with the dose of calcium carbonate in antacid suspension preparations, namely 1,250 mg/day. The sample that has been weighed is added to 70 mL of distilled water and then stirred using a magnetic stirrer which aims to make it easier to dissolve the sample and heated at 37ºC ± 3ºC. Heating at this temperature aims to condition the sample so that it resembles the condition of the human body, then standardized HCl 1N is added, the purpose of giving acid is a simulation of excess human stomach acid, stir for 15 minutes right after titration using the previous NaOH 0.5 N it has been standardized until it reaches a stable pH of 3.5. At this pH, it is a simulation of the normal conditions of human stomach acid [15]. NaOH and HCl solutions are secondary standard solutions and have low purity so that the concentrations they have are less stable, which can decrease the longer the storage process takes. Thus, NaOH and HCl solutions must be standardized before being used with primary standard solutions which have a high level of purity and known concentrations [17].

Standardization of NaOH 0.5 N solution uses a primary standard solution of oxalic acid 0.5 N as an analyte and phenolphthalein (PP) as an indicator. This standardization was carried out 3 repetitions. After the titration, the results obtained for the volume of NaOH can be seen in Table 3 so that from the results obtained the actual concentration of NaOH can be calculated, which is equal to 0.4983 N, close to the concentration of the desired NaOH solution. Standardization of the HCl 1 N solution used a primary standard solution of Sodium Tetraborate (Borac) 0.1 N and Methyl Orange as indicators. This standardization was carried out 3 repetitions. After titration, the results obtained for the volume of HCl can be seen in Table 4. So that the results obtained can be calculated the actual concentration of HCl, which is equal to 0.9866 N according to the desired HCl solution. The standardized values of NaOH and HCl that have been obtained are included in the calculation of the ANC (Acid Neutralizing Capacity) value by subtracting the moles of excess HCl added by $CaCO_3$ from the quail eggshells with the moles of NaOH needed during the titration. From this, we can obtain excess excess acid which is the neutralizing capacity of the $CaCO_3$ of quail egg shells [15]. The ANC value obtained can be seen in Figure 6.

Based on the results obtained, the ANC value of each fulfills the requirements because it is > 5 mEq which according to the FDA (Food and Drug Administration) requires the acid neutralizing capacity of antacids to be ≥ 5 mEq. The researcher also performed the ANC value on Calcium Carbonate Commercial (CCC) and obtained a ANC value of 24.40 mEq, based on data from quail egg shell ANC results, the highest ANC value was obtained in sample 5 ((≥100 mesh), which was 24.03 mEq, which means it is close to ANC CCC value. Looking at the results of ANC sequentially the smaller the particle size, the higher the ANC value, this is because the smaller the particle size, the larger the surface area, thus increasing the area in contact with the solvent. In addition, according to the $CaCO_3$ content in the quail eggshell, the highest content was owned by the sample that had the

smallest size, namely sample 5 ((≥100 mesh) with a $CaCO_3$ level value of 94.83%. This is directly proportional to the ANC, the higher the $CaCO_3$ content in quail egg shell powder, the higher the ANC value.

The size of the particle distribution can affect the value of ANC content in quail egg shells. To ensure a significant difference between each sample, an analysis was carried out using the SPSS One Way Anova and Post Hoc Test LSD (see the attachment). In the ANOVA test the data must be evenly distributed with a significant value (P> 0.05). The data used in ANOVA has a significant value of 0.495, meaning that the value is more than 0.05. This shows that the data is evenly distributed so that the One Way Anova test can be carried out. The results of the ANOVA test using the F test, obtained a calculated F value of 881,933 with a significant value of 0.00. Decision making is based on a comparison of F_{count} and F_{table}, if Fcount is smaller than F_{table} ($F_{count}<F_{table}$) then H_0 is accepted. From the ANOVA results, the calculated F value is greater than F_{table} (3.48), so H_1 is accepted and H_0 is rejected. This shows the influence of the size distribution of quail egg shell particles on ANC (Acid Neutralizing Capacity). The next test uses LSD, where the LSD test aims to see significant differences between each sample. LSD test results show a significant or significant difference if the value is significant (<0.05). And based on the data, each sample has a significant difference, sample 1 (20-40 mesh) has a difference with samples 2 (40-60 mesh), 3 (60-80 mesh), 4 (80-100 mesh), and 5 (≥100 mesh) as well as all other samples has a significant difference. This shows that there is an effect of the size distribution of quail egg shell particles on the acid neutralizing capacity because based on the LSD test all data from each sample has significant differences.

In this study, the speed of acid neutralization was carried out because the capacity of acid neutralization was not enough to see the effect of the particle size distribution of quail egg shells on acid neutralization. Besides that, the faster it can neutralize acid, the better the effectiveness of antacids. A sample of 1,250 mg was prepared and then a 250 mL beaker glass was put in, the number of samples used was in accordance with the dose of calcium carbonate in antacid suspension preparations, namely 1,250 mg/day. Samples were given 70 mL of distilled water, stirred using a magnetic stirrer at 37^{o}C and then given 1.25 mL of HCl 1 N solution. Check the pH every minute until the pH is stable after checking the last three times. When the pH is stable, it indicates that HCl 1 N has reacted completely with the $CaCO^3$ present in the sample. The results obtained can be seen in Table 5.

The results of the acid neutralization speed test for each sample sequentially, namely 23, 22, 20, 18, and 17 minutes, show that the smaller the particle size, the faster the acid neutralizes because at that time the pH was stable, where HCl 1 N had reacted perfectly. with $CaCO_3$ in the sample, while the larger the mesh size, the longer the sample can neutralize the acid because it takes a long time for the pH to stabilize. Sample 5 (≥100 mesh) is the fastest rate of acid neutralization compared to other samples because it only takes 17 minutes for the pH to stabilize, this is close to the CCC value of 15 minutes. Based on these data, it shows that the particle size distribution can affect the rate of acid neutralization. This is directly proportional to the acid neutralizing capacity, the smaller the particle size, the higher the ANC value and the faster the acid neutralization speed. On the other hand, the larger the particle size, the lower the ANC value and the slower the acid neutralization rate.

V. Conclusion

Based on the research that has been done, it can be concluded that there is an effect of the particle size distribution of quail egg shells on the acid neutralizing capacity, in which the smaller the particle size, the higher the value of the acid neutralizing capacity. The highest value of acid neutralizing capacity was found in sample 5 ((≥100 mesh), namely 24.03 mEq. And there is an effect of particle size distribution on the rate of acid neutralization, directly proportional to the ANC where the higher the ANC value, the faster the acid neutralizes. Sample 5 ((≥100 mesh) is the fastest sample to neutralize acid within 17 minutes.

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