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Original Article

Application of Partially Hydrolyzed of Virgin Coconut Oil (VCO) on Carrageenan-based Edible Coating as Fish ball Preservative

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Abstract: Food spoilage during storage may occur physically, chemically and biologically which is related to the activity of bacteria. One of the natural preservation that is currently developed is the application of edible coating on perishable food, such as fish ball. Addition of antibacterial agent is important to improve edible coating. Virgin coconut oil contains medium chain fatty acids which have antimicrobial properties, particularly monoglycerides and free fatty acids produced by hydrolyzing partially vco on the Sn-1 and Sn-3 positions using Lipozyme. The aim of this research was to study the effect of edible coating carrageenan enriched with hydrolyzed virgin coconut oil (HVCO) with concentrations 1%,3%,5% on fish ball quality. The samples were evaluated for 5 days at room temperature and analyzed for sensorial assessments, total plate count, total volatile base-nitrogen, water content, and pH. The study demonstrated that fish meatball coated with carrageenan based-edible coating fortified with HVCO showed the best result compared to controls (uncoated fishball, coated without HVCO). Sensory attributes were still accepted by panellists until 3 days. The same pattern depicted by TPC and TVB-N parameters. Edible coating with HVCO 5% inhibited microbial growth and retarded the increase of TVB-N in fish ball, withthe results were Log 5,08 cfu/g, and 29,69 mg/100 g respectively.

Keywords: Edible Coating Carrageenan, Hydrolyzed Virgin Coconut Oil, Fish ball

1. INTRODUCTION

The shelf life and quality of food during storage can be influenced by various factors, including microbiological damage by spoilage bacteria. Various methods are used to preserve food with physical and chemical methods such as the addition of chemicals as preservatives so that it may have a detrimental effect. One way to prolong the food quality that is currently being developed is the application of edible coatings. Edible coating is a thin coating material that is processed from biodegradable polymers as primary packaging of food with or without food additives [1]. Traditionally, edible coatings are used as food preservative from physical damage and microbial contamination. Edible coating technology has existed since the 12th and 13th centuries. At that time China had developed wax as a lemon coating to protect against microbial damage [2].

Hydrocolloids and food additives are the main ingredients in the preparation of edible coatings. One of the sources of hydrocolloids that are often used in edible coatings is carrageenan.

Carrageenan has good potential as raw material for edible coatings because it is elastic, inexpensive and renewable. Many researches have been conducted regarding the application of carrageenan-based edible coating to extend the self-life of food such as fillet, fish-ball, meatball, and fruits, but the carrageenan is bactericidal, the addition of anti-bacterial substances is the right solution to develop its role. One of them is the hydrolysis of virgin coconut oil (HVCO)[3-4].

Virgin coconut oil is dominated by medium chain fatty acids (MCFA) where lauric acid is the largest constituent in the range of 48%-53% [5]. Lauric acid and its monoglyceride, monolaurin, are very effective against bacteria, viruses and fungi. To produce monolaurin and free fatty acids is by hydrolysing partially acid lauric (triglycerides) either enzymatically, acidicly, or alkally. Enzymatic hydrolysis of VCO is more effective against bacteria than alkaline hydrolysis[6]. Therefore, HVCO which contains lauric acid and its monoglycerides are known to be potential as food preservatives like fish balls. The application of VCO on edible coating based-agar has been done for chicken ball preservation, but study on the application of HVCO on edible coating has never been conducted.

2. MATERIALS AND METHODS

2.1. Materials

The material used in this study was VCO (Viscoma) purchased from a drug store. The materials used for hydrolysis of VCO were virgin coconut oil (VCO), CaCl2 0.063M, Tris-HCL buffer solution, Lipozyme enzyme (Lipozyme@ TL IM). Prepation of fish balls, fish (*Sphyraena barracuda*) obtained from fish auction warehouses at fishing ports Tanjungbalai, North Sumatra, Indonesia. For the processing of edible coatings, the carrageenan used is semi-refined carrageenan from the Indoguna Indonesia brand. Reagents for analysis were plate count agar (Merck), perchloric acid (PCA) Merck, HCL (Merck), n-Hexane (Merck), Na2SO4 (Merck), KOH (Merck), phenolphthalein indicator.

2.2. Partial Hydrolysis of VCO

The VCO partial hydrolysis procedure was carried out according to [6]. A-50 gram of VCO was weighed in a 250 mL erlenmeyer, added with 50 mL of distilled water, 12.5 mL of 0.063M $CaCl_2$, 25 mL of 1M Tris-HCL buffer solution pH 8 and 500 mg lipozyme. The mixture was homogenized using a magnetic stirrer for 10 minutes. After homogeneous, the mixture was incubated for 10 hours at temperature of 50^{o}C and stirred for 10 minutes at a speed of 200 rpm every 1 hour. After the process is complete, the mixed solution is transferred into a separating funnel. Then extracted using 50 mL of n-hexane by shaking slowly. After that, the solution was allowed to stand until two layers were formed. The top layer (n-hexane fraction) is called filtrate 1. The bottom layer (water fraction) was extracted again with 50 mL of n-hexane, left it for a while, the top layer was separated named filtrate 2. The two filtrates were mixed and then added 50 grams of anhydrous Na_2SO_4 and left for 15 minutes to absorb the remaining water. Then filtered, then the n-hexane is evaporated using a water bath to produce hydrolyzed VCO (HVCO).

2.3. Preparation of Fishball

The processing of this fish ball is carried out in accordance with the procedure of SNI 7266, 2017 [7]. The first step is to prepare the raw material, namely Alu-alu fish (*Spyraena barracuda*). The first step is weeding. After that, washing and milling were carried out. The next step is grinding

and pulverizing. Then mixing spices and binders, namely 30% tapioca flour. After the dough is homogeneous and smooth, then moulding and boiling.

2.4. Preparation and Application of Edible Coating

The preparation of edible coating/film was carried out according to [8]. Provided 100 mL of distilled water into a 250 mL Erlenmeyer, added 1 gram of carrageenan. After that, homogenization was carried out and heated at 40^0C. Then the temperature was increased to 90^0C, stirred and 1% glycerol was added. After that, the temperature was cooled or decreased to 40^0C. Then 1% (B_1),3% (B_2) and 5%(B_3) HVCO were added, respectively. After that, apply to fish ball by dipping for 10 seconds, then drying. Dipped and dried fish balls stored at room temperature for five days and the quality then evaluated.

2.5. Acid Value Assay

Provided 5 grams of HVCO transferred into a 250 ml Erlenmeyer, 25 mL of 90% neutral ethanol was added. Heated in a water bath for 10 minutes while stirring. Then add 4-5 drops of pp indicator, after that it was titrated with 0.1 N KOH until the solution turned pink (the solution did not change for 15 minutes). The value of acid and free fatty acids of HVCO is calculated using the formula:

$$\text{Acid Value } = \frac{A \; x \; N \; x \; MW \; KOH}{w}$$

Where: (A= Volume titration of KOH), (N=Normality of KOH), (W=weight of sample), (MW= weight molecule of KOH).

2.6. Sensorial Assessments

Sensory testing is carried out by distributing score sheets (Indonesian National Standard, 7266, (2017) [7] and samples to 30 untrained panellists, panellists assessing the appearance, aroma, texture and taste of fish balls.

2.7. Total Plate Count (TPC)

The total plate count test was carried out by preparing the media according to [9], namely plate count agar (PCA) as much as 22.5 g and dissolved in 1 liter of distilled water, and prepare solution (0.9% of NaCl), after that it was sterilized in an autoclave at 121^0c for 15 minutes along with the equipment. After finished, prepare a sample of 10 grams, put it in a test tube and homogenize it with the diluents solution, called the first dilution, pour each 1 mL into a petri dish that has been filled with media, label it, repeat until the 5^{th} dilution. The sample was incubated for 24 hours at a temperature of 37^0C. After incubation, the number of bacterial colonies that grew in each dilution was calculated by the formula:

$$\text{Total Plate Count} = \frac{1}{dilute \; solution} x \; colony \; of \; bacteria$$

2.8. Determination of Total Volatile Base-Nitrogen (TVB-N)

The sample was extracted using a 6% perchloric acid solution. The extract was basicized by adding a 20% NaOH solution and then steam distilled, the distillate was accommodated in a 3% H_3BO_3 solution. The concentration of TVB-N in the distillate was determined by titration using a

0.02 N HCl solution, the method adopted from Indonesian Standard 2354, 2009 [10] and calculated by the formula:

$$\text{TVB (mgN/100gram)} = \frac{(Vc - Vb)x\ NHCl\ X\ 14{,}007X2X100}{W(gram)}$$

Where: (Vc=Volume titration of sample), (Vb= Volume titration of blanco), (NHCl= Normality of HCl), (14,007=Weight of nitrogen atom), (2= Diluent factor), (W=Weight of sample)

2.9. Content of Water

The procedure used to determine the moisture content by drying in an oven as described in (AOAC) [11]. Water content was determinate by the following equation:

$$\text{Water Content } = \frac{B-C}{B-A}x\ 100\%$$

Where: (A=weight of petri dish after drying), (B=Weight of petri dish and sample),(C= weight of sample and petri dish after drying)

2.10. pH Measurements

The sample was weighed 5 g, mixed with 50 ml of distilled water, blended and then filtered. After that, the electrodes were rinsed with distilled water and dried. The electrode is dipped in the filtrate for a while, until a stable reading on the pH meter is obtained, then the pH of the sample can be recorded [11].

3. RESULTS AND DISCUSSIONS

3.1. Acid Value

The results of the calculation of the acid number of VCO and HVCO produced in this study were as follows:

Table 1. Acid Number of VCO and HVCO

Sample	Acid Value (mg KOH/g oil)
VCO	1,344
HVCO	133,84

The results of the calculation of the acid value of VCO and HVCO obtained in this study were 1.344 mg KOH/g and 133.84 mg KOH/g oil respectively. Acid value of VCO is to indicate the free fatty acid content in 1 gram, whereas in HVCO is to indicate that is half of saponification value of VCO that is 250-260 mg KOH/g oil. The results of the acid value obtained from enzymatic hydrolysis (Lipozyme@ TL IM enzyme were almost the same as the results of the study Sipayung et al,(2019) [12]using the same incubation time of 10 hours.

3.2. The Effect of Carrageenan-based Edible Coating enriched with HVCO (1%,3%,5%) on Fish ball Sensory Attributes (Appearance, Odor, Texture, Taste)

The results of the sensory assessment which included appearance, aroma, texture, and taste by 30 untrained panellists on *Sphyraena barracuda* fish balls obtained the average results in the following figures.

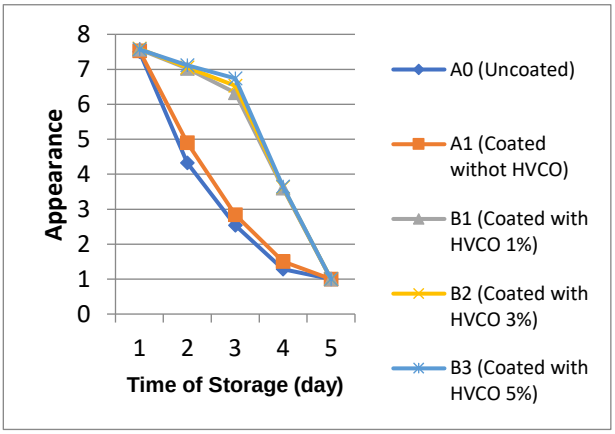

Figure 1. The effect of various treatments and storage time on fish ball's appearance

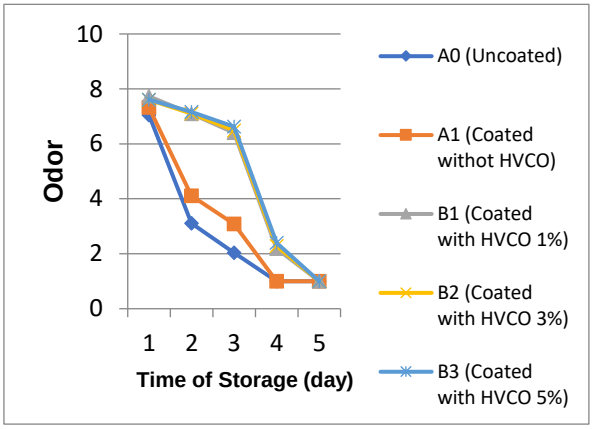

Figure 2. The effect of various treatments and storage time on fish ball's odor

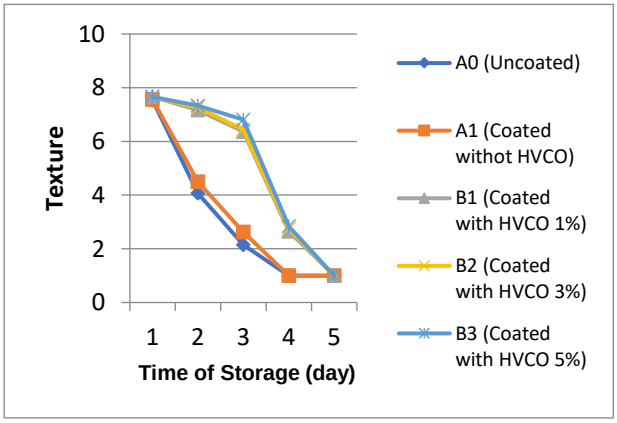

Figure 3. The effect of various treatments and storage time on fish ball's texture

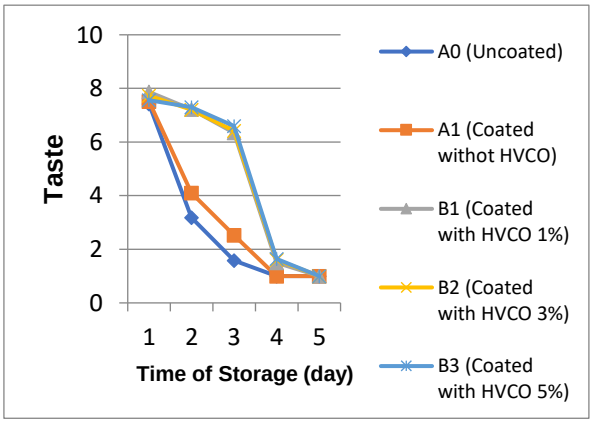

Figure 4. The effect of various treatments and storage time on fish ball's taste

3.2.1. Appearance

From Figure 1 it is depicted that in general on day 1 the panellists stated that they liked the appearance or appearance of the pestle fish ball (*Sphyraena barracuda*) and according to the minimum standard of SNI 7266 (2017), Results of the analysis of variance (ANOVA) conducted It is known that the P value < 0.05 at the 95% confidence level.

On the 2nd and 3rd days of storage, the appearance value of the pestle fish balls was still acceptable organoleptically, especially the 5% HVCO treatment, but the control had decreased in value. This is in accordance with research from [13] which stated that the appearance of catfish meatballs coated with carrageenan edible coating added with sesame oil on day 2 was still organoleptically accepted compared to controls.

3.2.2. Odor

From Figure 2, it is depicted that in general the panellists liked the aroma of fish balls on the 1st day of storage. Interestingly, the panellists stated that the meatballs coated with carrageenan-based edible coating enriched with 1% HVCO gave a distinctive aroma, where 1% HVCO could disguise the fishy aroma of the fish ball so that the panellists liked the most.

On the 2nd day of storage, the three treatments, namely B_1, B_2, B_3 decreased in value more slowly than the controls. Damage to the sample can be minimized by edible coating with the addition of HVCO so that it slows the growth or activity of microorganisms that can cause an unpleasant aroma in fish balls. In contrast to the control which was not received organoleptically from the 2nd day to the 5th day of storage. This occurs due to enzymatic and microbiological damage that causes the formation of trimethyl amin (TMA), thereby increasing the amount of Total Volatile Base Nitrogen compounds in fish balls which causes smell bad [14].

3.2.3. Texture

In general, the texture of all treatments on the 1st day of storage was favored by the panellists. The results of the analysis of variance (ANOVA) that were carried out showed that the P value <0.05 at the 95% confidence level, it can be concluded that the edible coating treatment with the addition of HVCO with various concentrations hit a significant effect on the texture of fish balls (*Sphyraena barracuda*) during storage.

On the 2nd day to the 5th day of storage, it was found that a very drastic decline value occurred in the control, where the texture in the control had started to become soft with a surface coated with mucus and had a sour taste, gelling, and emulsion have been damaged, so that the process of microbiological damage to fish balls will take place more quickly [15].In contrast, the fish ball coated with carrageenan edible coating added with 1%,3%,5% HVCO was able to maintain the texture until the 3rd day due to the inhibitory properties of edible coating and monolauric compounds in HVCO able to maintain protein functional properties such as protein binding capacity to water which prevents enzymatic and microbiological damage. This is the same as the research of Fangfang et al, 2019 [16], that the potato starch edible coating added with VCO was able to inhibit the growth of pathogenic bacteria.

3.2.4. Taste

On day 1 the panellists liked the taste of fish balls (*Sphyraena barracuda*) in all treatments, interestingly the panellists said that the addition of 1% HVCO was able to enrich the taste of fish balls and reduce the fishy aroma. On the 2nd day to the 5th day of storage, the taste of control decreased drastically, while the fish balls coated with a carrageenan-based edible coating with the addition of HVCO were able to last up to the second day with a value according to SNI 7266 min 7, while on the third day, the average value is below 7 but the category was still acceptable, the ability to maintain taste values in treatments B_1, B_2, B_3 because of the role of edible coating with the addition of HVCO which is able to inhibit the triggers of damage ranging from chemical damage to microbiological damage during storage at room temperature.

3.3. The effect of HVCO Treatments on Quality of Fish ball (TPC, TVB-N, Water Content, pH)

The results of testing on the quality of fish balls with different treatments which include the values of TPC, TVB-N, Water Content, and pH are shown in the following tables.

Table 2. The effect of HVCO treatments on Total Plate Count (TPC).

Treatments	**Storage of Time (day)log cfu/g**				
Concentrations	**1**	**2**	**3**	**4**	**5**
A_0	2.50±0.007^a	6.07±001^a	6.31±0.004^a	7.29±0.009^a	7.5±0.00^a
A_1	2.49±0.007^a	5.71±0.01^b	6.01±0.009^b	7.04±0.002^b	7.31±0.007^b
B_1	2.48±0.02^a	4.71±0.006^c	5.70±0.01^{cd}	6.31±0.01^c	6.96±0.006^c
B_2	2.50±0.02^a	4.47±0.003^d	5.65±0.01^d	6.22±0.002^d	6.80±0.01^d
B_3	2.50±0.007^a	3.00±0.012^e	5.08±0.004^e	5.74±0.007^e	6.5±0.04^e

Notes: A_0 = Uncoated, A_1= Coated without HVCO, B_1= Coated with HVCO 1%, B_2= Coated with HVCO 3%, B_3= Coated with HVCO 5%. The data is the result of the average value of three repetitions ± Standard of deviation. $p<0,05$.

Table 3. The effect of HVCO treatments on Total Volatile Base-Nitrogen (TVB-N)

Treatments	**Storage of Time (day)mgN/g**				
Concentrations	**1**	**2**	**3**	**4**	**5**
A_0	10.44±0.02^a	38.28±0.31^a	41.83±0.32^a	45.00±0.32^a	50.60±0.32^a
A_1	10.42±0.09^a	36.78±0.32^b	40.15±0.33^b	43.32±0.32^b	48.36±0.32^b
B_1	10.38±0.06^a	24.84±0.6^c	30.25±0.00^c	40.15±0.32^c	43.71±0.02^c
B_2	10.42±0.02^a	23.72±0.3^d	30.33±0.5^c	39.26±0.09^d	42.61±0.06^d
B_3	10.38±0.02^a	22.60±0.34^e	29.69±0.00^d	38.83±0.32^d	41.46±0.00^e

Notes: A_0 = Uncoated, A_1= Coated without HVCO, B_1= Coated with HVCO 1%, B_2= Coated with HVCO 3%, B_3= Coated with HVCO 5%. The data is the result of the average value of three repetitions ± Standard of deviation. $p<0,05$.

Table 4. The effect of HVCO treatments on water content

Treatments	**Storage of Time (day) (%)**				
Concentrations	**1**	**2**	**3**	**4**	**5**
A_0	62.00±0.00^a	65.33±0.57^a	67.33±0.57^a	68.50±0.00^a	72.66±0.57^a
A_1	62.00±0.00^a	64.50±0.00^b	66.00±0.00^b	68.00±0.00^b	71.67±0.57^b
B_1	61.83±0.28^a	63.66±0.57^c	65.16±0.76^c	67.60±0.57^c	68.16±0.2^c
B_2	61.50±0.86^a	62.83±0.3^d	65.00±0.00^c	66.67±0.57^d	68.00±0.00^{cd}
B_3	61.66±0.57^a	62.00±0.00^e	64.67±0.57^c	65.83±0.3^e	67.67±0.57^{de}

Notes: A_0 = Uncoated, A_1= Coated without HVCO, B_1= Coated with HVCO 1%, B_2= Coated with HVCO 3%, B_3= Coated with HVCO 5%. The data is the result of the average value of three repetitions ± Standard of deviation. $p<0,05$.

Table 5. The effect of HVCO treatments on pH value

Treatments	Storage of Time (day)				
Concentrations	**1**	**2**	**3**	**4**	**5**
A_0	6.80±0.003^a	6.65±0.00^a	6.59±0.006^a	6.50±0.005^a	6.38±0.005^a
A_1	6.78±0.006^a	6.66±0.005^a	6.60±0.005^a	6.52±0.00^a	6.40±0.005^a
B_1	6.80±0.004^a	6.70±0.005^b	6.63±0.00^a	6.60±0.005^b	6.52±0.005^b
B_2	6.78±0.01^a	6.71±0.005^b	6.65±0.006^a	6.60±0.00^b	6.53±0.005^b
B_3	6.78±0.005^a	6.72±0.00^b	6.69±0.00^a	6.63±0.006^b	6.60±0.005^{bc}

Notes: A_0 = Uncoated, A_1= Coated without HVCO, B_1= Coated with HVCO 1%, B_2= Coated with HVCO 3%, B_3= Coated with HVCO 5%. The data is the result of the average value of three repetitions ± Standard of deviation. $p<0,05$.

3.3.1. Total Plate Count (TPC)

From the results of the total plate count (TPC) calculation that has been carried out, it is known that the total bacteria in each treatment was still under threshold on the 1st day of storage, but on the 2nd to 5th day of storage, the increase in the number of microbial contamination of control is very drastic, the number reached the maximum limit of microbial contamination allowed by Indonesian Standard, which was $5x10^5$ (Log 5.70 cfu/g).

In treatments of B_1(HVCO 1%), B_2 (HVCO 3%), B_3 (HVCO5%), the increase in the amount of microbial contamination on the 2nd day of storage was not as fast as the control treatment because the monolaurin content in HVCO, Monoglycerides and MCFA in VCO broke down bacterial membranes with the help of gastrointestinal lipase enzymes. The working system of MCFA, especially lauric acid, changes the permeability of the bacterial cell wall, interferes with metabolism, inhibits important nutrients needed by bacteria or is associated with carbohydrate metabolism.5% HCVO treatment is still according to Indonesian national standards until the 3rd day of storage [17].

3.3.2. Total Volatile Base-Nitrogen

From the results of the tests carried out, it is known that the TVB-N value of fish ball (*Sphyraena barracuda*) on the 1st day of storage is still in accordance with SNI, ECC and Farbers standards 35 mg-N/100g, 30 mg-N/100g, 20<TVB-N<30mgN/100g respectively [22] stated that the TVB-N value increased with increasing storage time. However, a significant increase occurred in the control treatment until 5th day storage.

The quality of fishery products such as fish balls is mostly related to changes in chemical composition and degradation of muscle protein during the cooking and storage process. Enzyme action present in fish products or microbial activity, nitrogen compounds such as tri-methylamine-oxide (TMAO) are degraded into ammonia, di-methylamine (DMA), formaldehyde and tri-methylamine (TMA), with increasing bacterial spoilage, there is an increase in the breakdown of TMAO into TMA and DMA [14].

3.3.3. Water Content

The increase in water content in the control was caused by the absence of a barrier such as coated fish balls. In addition, according to [18] during storage, the increase occurred due to the

protein denaturation process so that it lost its ability to bind water. The same results were also reported by [19] the water content of fish meatballs coated with bio-composite edible coatings tended to increase more slowly than controls stored at room temperature.

3.3.4. pH Value

From the results of pH tests that have been carried out on pestle (*Sphyraena barracuda*) fish balls with different treatments, the results were not significantly different on the 1st day of storage. Along with the length of storage, it has a significant effect on the pH value of the fish ball. The result of analysis of variance showed that $p<0,05$.

The decrease in pH during storage occurred in the treatment and control, but a faster decrease occurred in the control until the 5th day of storage. The pH value can change due to the activity of microorganisms that degrade carbohydrates into simpler compounds (lactic acid, acetic acid) [20]. In addition, according to [21] the breakdown of carbohydrates in foods will cause softening to form acidic compounds that can lower the pH value, then gases are formed which affect the taste.

4. CONCLUSION

The application of edible coating carrageenan enriched with HVCO on (*Sphyraena barracuda*) fish balls had increased and maintained the sensory attributes during storage at room temperature, where it was known that treatment B_1 (1%) was preferred by panellists compared to other treatments in terms of taste and aroma. The treatment of B_3 HVCO (5%) still persisted and was preferred by the panellists until the 3rd day of storage. The concentration of HVCO in the edible coating of carrageenan which was applied to the fish ball was able to maintain quality during storage at room temperature. The higher the concentration of HVCO in edible coating the better the quality of fish ball during storage at room temperature

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