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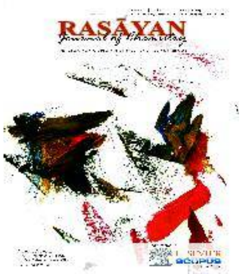

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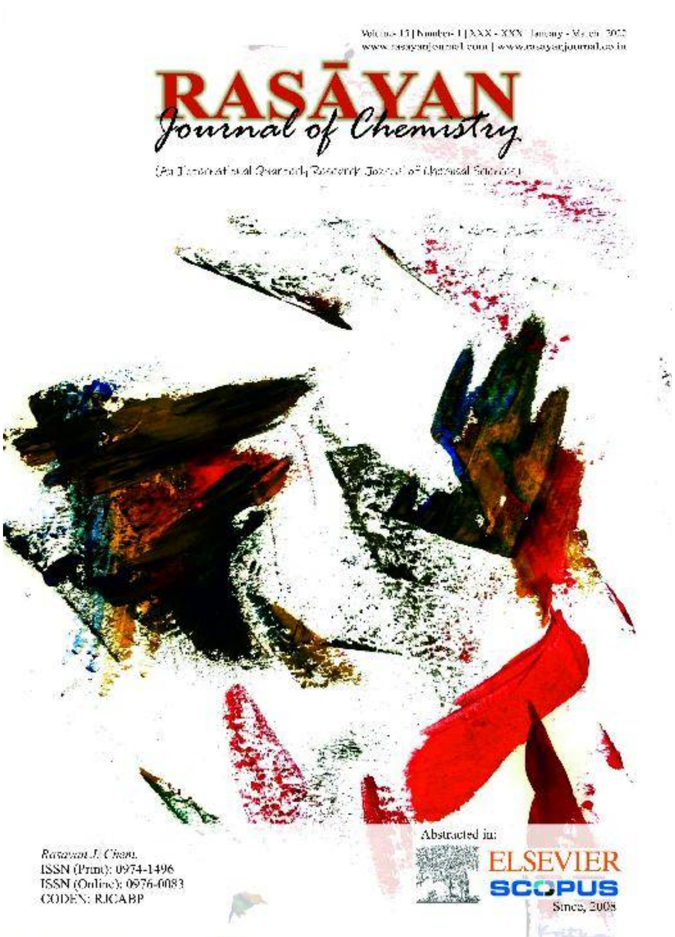
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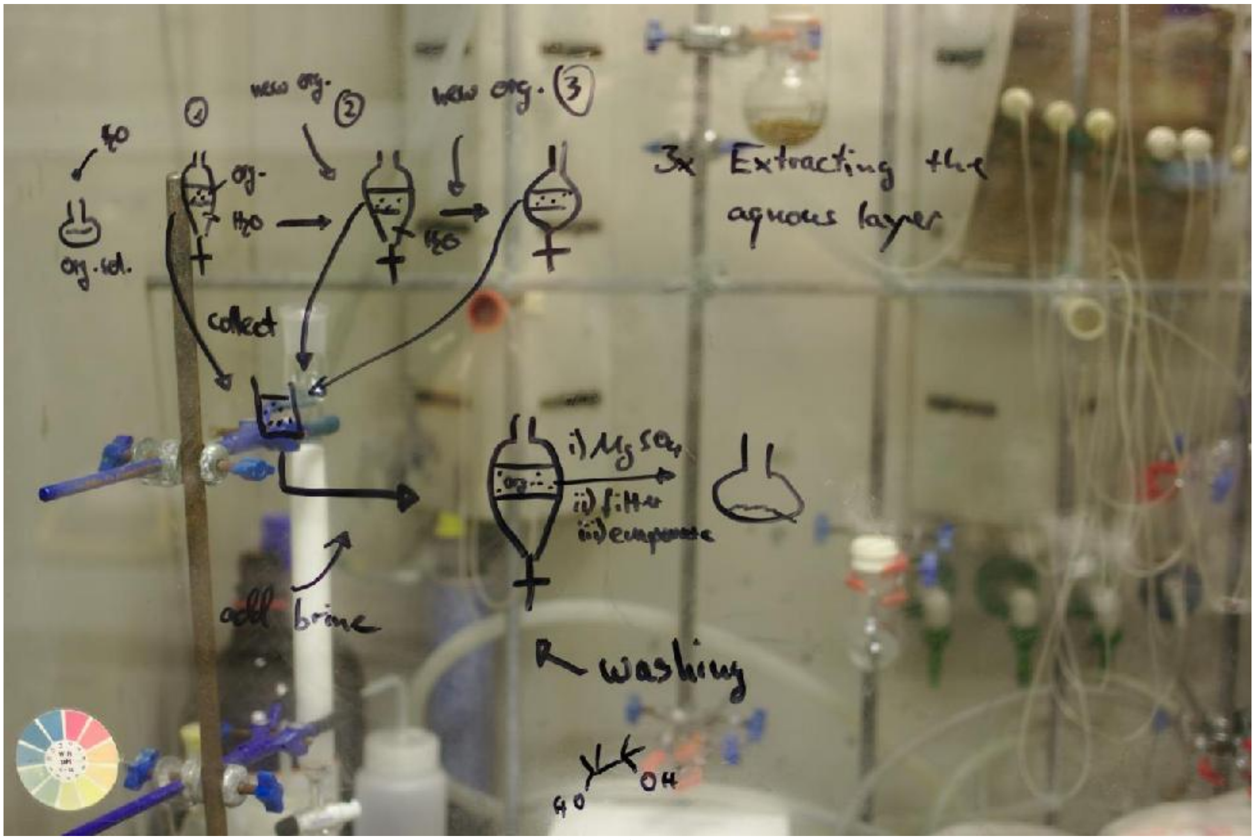

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THE EFFICACY OF GINGER ESSENTIAL OIL WITH CHITOSAN TO VIABILITY of *Staphylococcus aureus* IN FRUITS

Flora Elvistia Firdaus✉, Adam Saputra and Anisfa Neva Wardana
Department of Chemical Engineering, Jayabaya University, Jakarta 13210, Indonesia
✉Corresponding Author: flora_elvistia@yahoo.com

ABSTRACT
Staphylococcus aureus was one type of bacteria most commonly found in the human skin. Direct contact with fruit skin can effected to rotten. It was found the increasing use of synthetics materials for foods preservative. The replacement of synthetics with natural alternatives would certainly solve the current demand for safe food packaging. The films on the packaging were manufactured from natural white ginger essential oil (GEO) to be incorporated into chitosan film synthesized from whiteleg shrimp shells. The purpose was to attain a high degree of safety antimicrobial for fruits, specifically apples, and pears. The blends of GEO and chitosan were studied its effectiveness for the quality of product indicated by the population of the viable bacterial count, the inhibition of bacterial activity, and fruit weight loss. The design of this work was (chitosan: GEO) was 6:2 (w/w); 6:20 (w/w), and 6:24 (w/w). The existing low growth of microbial by 6:20 (w/w) and suppressing the weight loss of apples and pears. This can be concluded that GEO's blending to chitosan effectively on pathogenic Staphylococcus aureus gram-positive.
Keywords: Antimicrobial, Chitosan, Ginger Essential Oil, Films Packaging, Staphylococcus aureus.
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INTRODUCTION

The freshness of fruits is an important criterion that dictates consumer preference for buying better quality products. The biodegradable fruits are sensitive to decay due to microbial attacks, affecting low product quality. Acceptable fruit preservatives are needed[1]; they should be required safely for health.[2] Biopolymers are commonly used as an edible coating for fresh fruits to produce moisture and reduce oxidative reaction[3], and carrying antibrowning, colorants. Chitosan has a good ability of film-forming[4], reduce water loss by creating a semipermeable barrier[5], exhibit an antifungal and antimicrobial originated from its polycationic nature[6-8] to reduce pathogen growth on fruits surface.[9] The presence of charges in its backbone polymer forming an ionic interaction to bacteria cell wall causes microbial wall hydrolysis, cellular leakage, and microbial death.[10] The application of chitosan for active packaging can develop safety enhancement on food products. Chitosan is the second most abundant in nature after cellulose[11]. The main sources are found in crustaceans shells. The production cost is low compared to enzymatic processes, moreover if full production scale.[12] The partially deacetylated chitin into chitosan has made the positive charge of amino groups influence the antiviral activity[13,14], which inhibits the growth of bacteria and fungi.[15] Chitosan is categorized by the degree of deacetylation (DD), which is soluble in diluted acidic[16], organic acids[17], with the low condition, enable chitin synthesized from shrimp shell to obtain a high DD.[18]

Antimicrobial has specific inhibitory activity against microorganisms. The antimicrobials activity is suitable for bacteria of gram-negative and gram-positive[19], which had caused *Staphylococcus aureus* gram-positive bacterial impaired. Nevertheless, the role of chitosan was still facing much doubt because of some limitations on preserving fruit and more effectively inhibiting gram-negative bacteria.[20]

The efficacy of active substances in essential oil into chitosan film has demonstrated enhance the antimicrobial of chitosan on preserving fruits with a better moisture barrier by changing the cell membrane permeability.[21] The blending commonly as a hydrophilic matrix into a hydrophobic compound or vice versa, where the antimicrobials are often combined.[22] Moreover, current consumer demands on food safety and safer packages.[23]

The major function of active antimicrobial packaging was to build up a protective barrier against the invasion of microorganisms. This research aimed to determine the support of ginger essential oil to chitosan

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for antimicrobial packaging on pear and apple indicated by fruit weight loss, viable microbial count, and the antimicrobial inhibition for preliminary screening.

EXPERIMENTAL

Material and Methods

The fruits material of this work are pears *(Pyrus)* and apples *(Ma/us sylvestris mil/are)* are local fruits to be covered by antimicrobial film composite-based packaging. The pears were harvested in Cimanggis West Java. Indonesia, and soon handled to proceed to the laboratory. The total fruits were 24 apples and 24 pears, comprising 4 for control for apples and 4 for pears. Each of the formulae was done in duplicates. The fresh apples were purchased from the commercial market in East Jakarta, Indonesia, about 1.5 km away from the laboratory. The average weight of apples and pears were respectively 89.03 g and 264.90 g.

The antimicrobial composite was chitosan synthesized from a whiteleg shrimp shell performed as the matrice for ginger essential oil (GEO). The whiteleg shrimp shells were collected from the hotels' restaurant in central Jakarta and the fresh, natural ginger was purchased from the traditional market in East Jakarta. The research design was the mixture of chitosan 2% (w/v) and 0.5% (w/v) GEO with the formula 6:2 (w/w); 6:20 (w/w), and 6:24 (w/w).

Manufacture of Ginger Oil

Preparation of ginger essential oil. The natural ginger was conducted the maceration method with the process without heating. The principle of the maceration method is the occurrence ofleaching on the active component in the sample. The longer the contact time, the more secondary metabolites will be extracted. The natural ginger was washed out from the soil using running water in this research. Cut into small pieces, dried in the direct sun for 3-4 days to obtain completely water-free, then mashed using a blender. Weigh the ginger Simplicia. immersed in methanol 98%. Weigh the filtrate. Soak again until the result obtained was a clear color filtrate. Furthermore, using a rotary evaporator for solvent evaporation resulted in a concentrated filtrate and continued using a water bath until it reached a more concentrated solution approximately 100/ovolume from the previous.

Manufacture of Chitosan

The procedure of making chitosan in this research was adopting the works of Younes *et.al* [12]. The chitosan was synthesized from whiteleg shrimp shell. The shell was washed using running water and dried in the direct sun for two days. Mashed the shell using a blender, sifted with 50 mesh. The dried shrimp shells were carried out to the deproteination process, which aimed to breakage the bond of protein and chitin. The product was then deminemlized to remove the minerals; $CaCO_3$ and Phosphor to produce chitin. The chitin obtained is deacetylated by adding concentrated sodium hydroxide to remove the acetyl group ($NHCOCH_3$) to become an amine group.

Deproteination

The principle of deproteinization is disrupting the bonds of chitin with proteins. NaOH is the preferential reagent beside a wide range of other chemicals Na_2CO_3, $NaHCO_3$, KOH, K_2CO_3, $Ca(OH)_2$, Na_2SO_3, $NaHSO_3$, $CaHSO_3$, Na_3PO_4. and Na_2S. The concentration ofNaOH is in the range from 0.125 to 5.0 M, It can partially deacetylate the chitins and hydrolyze the biopolymers. In this research, the fine shrimp shell was added a 3.5% NaOH solution with a ratio (1:10) (w/v). Stirred and heated to a temperature of 65 °C for 2 hours. Cool and strain, and wash with distilled water. The filter is put into an oven with a temperature of 60 °C until dried.

Demineralization

The principle of demineralization is removing minerals; originally $CaCO_3$ was generally performed by acid treatment using HCI, HNO_3, H_2SO_4, CH_3COOH, and HCOOH. The preferential reagent is a dilute hydrochloric acid, with the reaction:

$$2\,HCl + CaCO_3 \rightarrow CaO_2 + H_2O + CO_{2\,(g)}$$

Since heterogeneity of the solid is so arduous to release the minerals, some modifications of native chitin such as deacetylation and depolymerization can be attained by high concentrated acid, like 900/0 formic acid and 22% HCl can be used for demineralization.[18] Afterward, methods have been developed to minimize degradation with no residual ash. A mild concentration of HCl is preferential with a slight drop of ash. The extraction of chitin from shrimp shells using mild conditions by 0.25 M HCl in the stoichiometric amount. In this research, the deproteinated shrimp shell in powder was done by adding 1N HCl solution with the ratio (1:15) (w/v). Stir and heat at 40 °C for 30 minutes. Cool, then strain, and wash with distilled water. The filter results were put in the oven at a temperature of 60 °C until dried.

Manufacture of Chitosan-GEO Films for Fruits Application

According to some references, the acetic acid used for diluted chitosan can be ranging from 0.05 to 0.1% (v/v). [6] The chitosan prepared for this work was 2% (v/v) by weighing 10 g of chitosan, then added a 2% (v/v) acetic acid until it reached the volume scale of 500 mL, the ginger dissolved in aquadest to be prepared for 0.5% (w/v), and the mixture homogenized. The chitosan was then enriched with 0.5% (w/v) ginger essential oil (GEO), forming a composite. The chitosan was performed as a matrice to GEO under room temperature. The ingredient of the composite was prepared in three formulas with each of them in duplicate. The first formula was chitosan: GEO with the ratio of 6:2 (w/w) where the composite was prepared for the weight of 500 g. The procedure was by weighing the chitosan 2% for 333 g and weighing 167 g of 0.5% GEO then homogenized. The second formula was the preparation of 2% chitosan enriched with GEO 0.5% with a ratio of 6: 20 (w/w) for 500 g of the weighted composite. The step for the weight results expected, by weighing 2% chitosan solution for 115.4 g, then add to 384.6 g of 0.5% GEO, then homogenized the mixture. Lastly, the preparation of 2% chitosan and GEO 0.5% with a ratio of (6:20) (w/w) with 0.5% GEO for a 500 g of the weighted composite. The steps weighed 100 g of 2% chitosan solution, then weighed 400 g of 0.5% GEO, and then homogenized the mixture.The 20 x 20 cm filter paper were dipped into different beaker glass filled with : 2% chitosan solution, 0.5% GEO solution, and mixture of 2% chitosan: 0.5% ginger extract (6:2; 6:20; 6:24) (w/w). This was aimed to absorb the active substance of the preservatives, let it dry naturally at room temperature. Wrap the filter paper to apples and pears. Each of the tests was done in Duplo.

Degree of Deacetylation (DDA)

The degree of deacetylation (DDA) is N-acetyl group removal in chitin to partly deacetylated to amino, transforming chitin into chitosan in the deproteination stage. The value of DDA was associated with solubility. Acids and alkalis can be used in the process of deacetylation, but the fact the glycosidic bonds are more susceptive to acid, so alkali are become more frequently used. [25] The addition of 50% NaOH, the acetyl group was hydrolyzed from the acetamide group in chitin. FTIR spectrophotometer was used to determine the DDA where the hydroxyl group was at a wavelength of 3450 cm^{-1}, amide groups at 1655 cm^{-1}. The value of chitosans (DDA 50% or DA 50%) was more water-soluble, while chitosan with DDA > 60% or DA < 40%) was soluble in acids.[12, 26,27]

In this research, the demineralized shrimp shell was added to a 60% NaOH solution with a ratio (1:20) (w/v). Stir and heat at 100 °C for 1 hour. Cooled, then filtered and washed with aquadest. Filter results, put in an oven with a temperature of 80 °C until dried.

Evaluation of Antimicrobial Activity by Paper Disc Diffusion

The paper disc diffusion method measured the antimicrobial activity alone or incorporated in the form of coating, packaging, and film. This aim was for the preliminary screening test, which was based on the inhibition zone around the paper disc. The principle of this method was to determine the antimicrobial activity by observing the clear zone, which indicates the presence of microbial growth inhibition by antimicrobials on the surface of agar media.[28]

A 1-2 ose of REMEL strain *Staphylococcus aureus* ATCC BAA-1026 was suspended with sterile physiological sodium chloride physiological solution 0.85 % (Merck). The 1 mL of suspension was inoculated into a duplicate nutrient agar (Sigma-Aldrich) media in a petri dish. The agar media were previously thawed with a temperature of 45 ±1°C then poured and gently shaken, allowed to solidify. Prepared the preservative sample solution which were: 2% pristine chitosan, 0.5% pristine GEO, blending 2% pristine chitosan and 0.5% pristine GEO (6:2 ; 6:20; 6:24) (w/w). Dipped the disc paper using aseptically tweezer in 70% alcohol into the sample solutions, then put into the mixture of nutrient agar media and *S aureus*. Incubated at 35°C for 24 hours, it was done in duplo. Measure the clear zone around the paper disc in millimeters (mm).

Determination of Antimicrobial Effectiveness by The Total Plate Count

The principle of the total plate count (TPC) method was based on all the living cells that will develop into colonies.[29] Weighed 25 g of a sample into an Erlenmeyer flask containing 225 mL of diluent solution (1:10) (w/v). Homogenized the sample, make a serial dilution 10^{-1}; 10^{-2}; 10^{-3};10^{-4}, and 10^{-5}. Pipette 1 mL from each dilution into a duplicate sterile petri dish, as well as media blanks sample and solvent. Each petri dish was poured a 12 mL of melted plate count agar (PCA) media with a temperature of 45 ±1°C within 15 minutes from the first dilution, then homogenized, then germinated. During incubation, the petri dish was placed upside down with the temperature maintained at 35 ± 1°C for the duration of 24-48 h. The colony growth was recorded. The TPC was counted, for 1 mL of the sample multiplied the average number of colonies with the inverse dilution factor. The samples were prepared in two repetitions. It was observed and calculated the average on day-1 (24h and 48h) and day-12 (24 h and 48h).

Weight Loss Test

The chitosan – GEO as an antimicrobial film for packaging was performed as an oxygen barrier, where the absence of the oxygen can extend shelf life. Some external factors related to the growth of the microbial such as moisture, the non-oxidative reaction, enable some enzymatic activity were individually or collectively deteriorate food-product and causing weight loss, and affect the quality and appearance of the fruit.

Several studies mentioned the ability of chitosan to successively reduce fruit spoilage by lowering the rate of respiration, inhibiting mold growth which means inhibiting ripening by reducing ethylene and carbon dioxide production. Chitosan film enabling inhibits psychotropic pathogens. According to research conducted by Bai *et.al,* the chitosan coating (2% chitosan in 5% acetic acid) was able to inhibit the decrease of anthocyanin content and increase polyphenol activity oxidase during lychee storage. [30] Referred to Gaouth's findings, the chitosan coatings (1% and 2% in 0.25 N HCl) reduce respiration rate and ethylene production of tomato, where physically the organoleptic test on chitosan-coated tomatoes was harder, and less red pigmentation than controls after 4 weeks of storage at 20^0C.[31]

The weight loss of fruits was considered, it reflected too strongly related to the enzymatic reaction indicated to fruit skin texture. The weight loss was calculated by weighing fruits before and after adjusting to the days designated, the results of the weight loss were presented in percentages (%). Each sample was performed in two repetitions.

RESULTS AND DISCUSSION

Chitin obtained was a reddish-white powder that had the aroma of a little fishy, after becoming chitosan was turned into white and odorless powder.

The Degree of Deacetylation (DDA)

The DDA was determined by the absorption spectra of FTIR spectroscopy. The comparison of amide band absorption (1655 cm^{-1}) to hydroxy band absorption (3450 cm^{-1}). The high degree of DDA and low acetyl group indicated that the properties of chitosan are close to ideal homopolymers. In this research, the value obtained was (DDA 66.68% or DA 33.32%). The degree of DDA was the main factor for measuring the quality of chitosan. The higher degree of the DDA, the superior property to chitosan obtained, includes the solubility, the antimicrobial purposes, etc.

Microbial Inhibition

To visualize the formation of a zone of inhibition of the antimicrobial on *Staphylococcus aureus*, the inhibition zones were measured based on the average diameter of the clear area on Petri dishes.[12,14,32,33] The Duplo replicate plates were used for each concentration and data were subjected to statistical by one-way analysis of variance (ANOVA) with the significance $p \leq 0.05$. The observed inhibition zone of the antimicrobial on *Staphylococcus aureus* has shown that the formula 6:20 (w/w) was the highest radial compared to chitosan: 6:24 (w/w) and chitosan: 6:2 (w/w).

The optimized inhibition of *S aureus* to chitosan: GEO (6:20) (w/w) was 10 ±1.2 mm, while the optimized inhibition of (6:24) (w/w) was 8 ±1.08. The optimized of pristine chitosan was 20 ±1.67 mm and the optimized of pristine GEO was 17 ±1.47 mm. These steps were taken as a screening test before being applied to apples and pears to determine which formula of the preservatives chitosan: GEO was the best. The images' inhibition of preserving pears and apples can be seen in (Fig.-1).

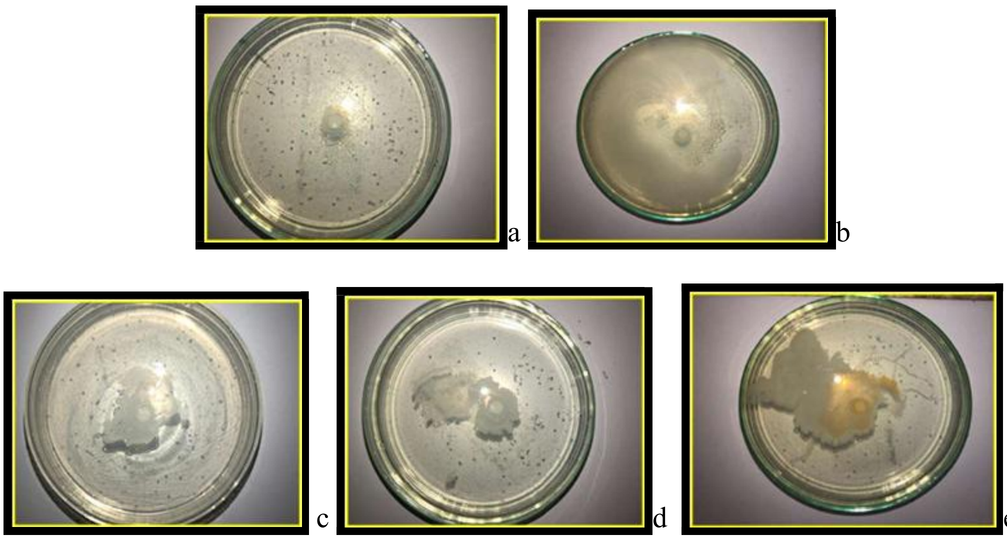

Fig.-1: The Images of antimicrobial inhibition on *Staphylococcus Aureus* by chitosan: GEO was meant as the screening test before being applied to apples and pears. (a) 10 ±1.2 mm (6:20) (w/w) ; (b) 5 ± 0.08 mm (6:2) (w/w) ;(c) was 8 ±1.08 (6:24) (w/w); (d) pristine chitosan 20 ±1.67 mm and (e) pristine GEO 17 ±1.47

Microbiological Parameter by Total Plate Count

The semipermeable barrier created by chitosan is useful for the extended shelflife of fruits, the hydrophobic essential oil extracts boosted 4 by its active volatile terpenes, terpenoid, and aromatic constituent.[34] Chitosan with essential oils has presented better incorporation.[35] The dispersion of chitosan in acid water solution 1 % acetic acetate resulted in the chitosonium acetate film matrix, this was the fact the solubility of chitosan was influenced by acetic acid in solution. The synergistic of chitosan with (DDA 66.68% or 33.32% DA) incorporated by GEO, with the task division gram-positive bacteria inhibits by chitosans and the gram-negative bacteria by GEO. The mixture successfully suppressed the microbial count even better than pristine GEO and was slightly similar to pristine chitosan. The incorporation of ginger essential oil (GEO) into the matrix was done at room temperature forming uniformity with no heating process. This can be further beneficial for fruit preservation. From the observations at predetrimental twelve-day intervals to apples and pears. The synergistic outcome of antimicrobial activity at a ratio of (6:20) has given an additive effect where the combining antimicrobial interaction was quite similar to pristine chitosan. The ratio of (6:2) and (6:24) has given the opposite result where the combining antimicrobial is much lower than pristine chitosan and even pristine GEO. The incorporation of GEO to chitosan can be optimized to be achieved in a homogenized miscible of composites chitosan-GEO, type of fruit preserved, and storage condition. Although some published works have raised discussion, the effectiveness of chitosan and its derivative on gram-negative and gram-positive bacterial was somewhat controversial. Several analyses emerged to the bacterial cell wall thickness predominant to the easily accessible on cell lysis, where gram-negative was thinner than a thick and rigid gram-positive cell wall (Fig.-2).

Apart from other factors that are quite important and very determining was the electrostatic interaction between the positive charge of chitosan and the negative charge of microbial cells [10]. Eventually, the ratio (6:20) applied for the active packaging of apples and pears proved the success of GEO on supporting chitosan which was indicated by lowering the growth of *Staphylococcus aureus,* a gram-positive bacterial. Based on the research findings, the application of pristine chitosan on apples and pears was more effective than pristine GEO in suppressing the growth of viable bacterial count. The fact of the inoculation of *S aureus* strains on the sample design, that does not mean it would be the domain of chitosan as commonly known works better inhibits the gram-positive bacterial as *S aureus* one of them. The fact the price of pristine chitosan was very costly, the use of cheap GEO could be taken into consideration, where the synergetic of both antimicrobials has been proven effective in inhibiting bacterial growth.

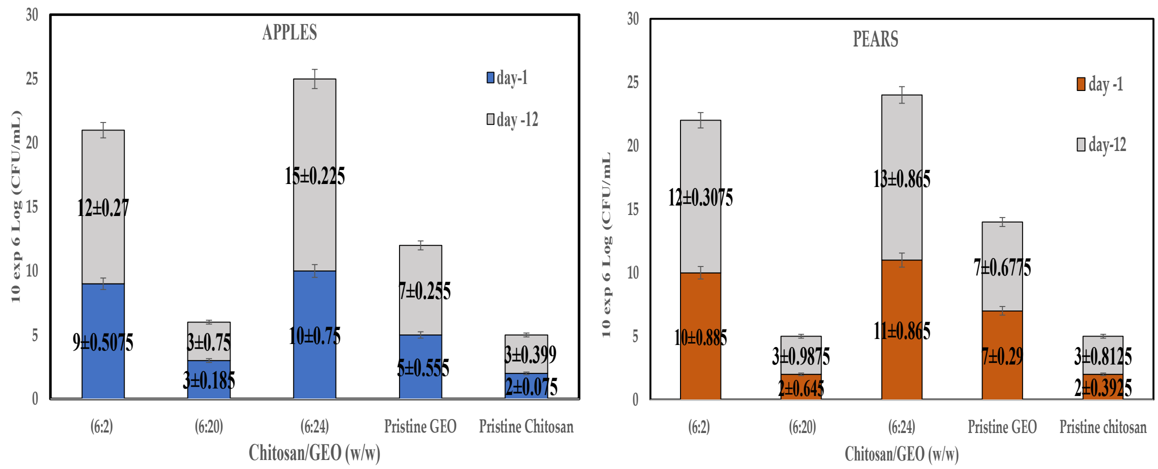

Fig.-2: The antimicrobial of chitosonium acetate – GEO hybrid against *Staphylococcus aureus* after incubation for 24 h day-1 and day-12. The cells in the exponential with initial inoculum approximately 10^6 CFU/ mL were employed in all ratios of the composite then applied to apples and pears in two repetitions. They were significantly determined by ANOVA with ($P \leq 0.05$).

Fruits Weight Loss

Weight loss tests can provide information to corroborate other test results. It appears that there was a tendency to increase the fruit weight loss according to the day function applicable to both fruits. The pears fruit weight loss was relatively higher than apples.

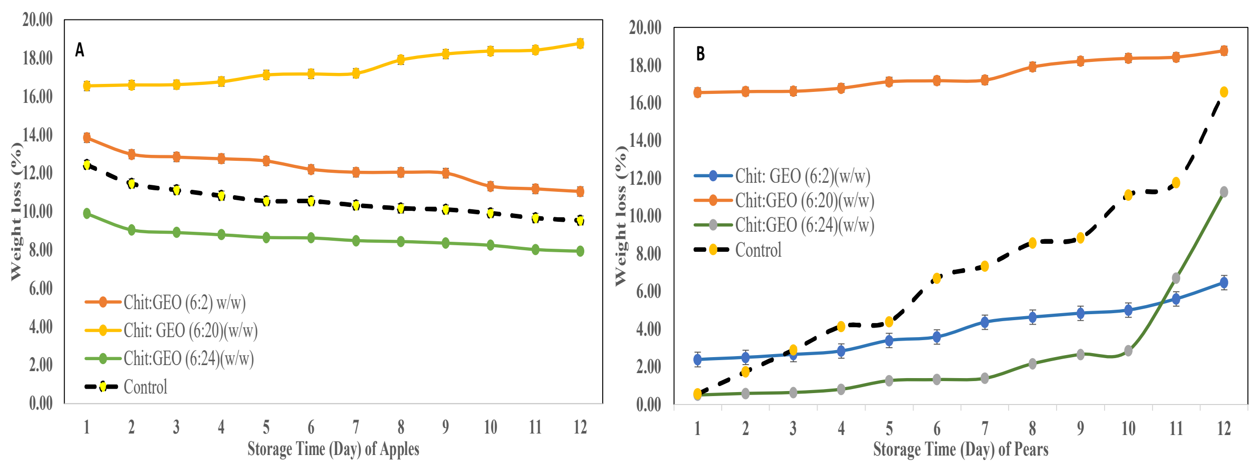

Fig.-3: Weight loss percentage (average ±SD) of 6:2 (w/w); 6:20 (w/w) and (c) 6:24 (w/w) and (d) No antimicrobial. Data were subjected to statistical evaluation by one-way analysis of variance (ANOVA). The significance $P \leq 0.05$. The observation was conducted in twelve days on (a) apples and (b) on pears. Each formula was done in duplicate.

Referred to the finding of the work of Abdollahi where the addition of essential oil to chitosan forms a hydrophobic interaction reduced the water vapor and moisture[36], the use of GEO in the chitosan formula successfully lowering the weight loss of apples and pears where the (6:20) was better than (6:2); and (6:24) w/w, where the slope was slightly increased. Even though the (6:24) were previously low at the first ten days, but it continued to rise after day-10 until day-12 of observations. The result on pears was more excellent than apples (Fig.-3).

The summary of the antimicrobial activity of chitosan-GEO against *Staphylococcus aureus* was presented in (Table-1).

Table-1: Summary Antimicrobial Activity of Chitosan- GEO against *Staphylococcus aureus*

Antimicrobial for *Staphylococcus aureus*						
Apple		(6:2)	(6:20)	(6:24)	Pristine Chitosan	Pristine GEO
	Highest weight LossDay-129%)	11.05	7.94	18.77	NI	NI
	Viable Count Day-12(Log CFU/mL)	12±0.27	15±0.225	3±0.75	3±0.399	7±0.255
	Gaining of Viable Count (log CFU/mL)	2±0.7625	NI	0.565	1±0.7	1±0.324
	Reduction of Viable Count(Log CFU/mL)	NI	0.565	NI	NI	NI
Antimicrobial for *Staphylococcus aureus*						
Pears		(6:2)	(6:20)	(6:24)	Pristine Chitosan	Pristine GEO
	Highest weight LossDay-129%)	6.46	11.26	18.77	NI	NI
	Viable Count Day-12(Log CFU/mL)	12±0.3075	3±0.9875	13±0.865	3±0.8125	7±0.6775
	Gaining of Viable Count (log CFU/mL)	1±0.4225	2	NI	0.3875	1±0.42
	Reduction of Viable Count(Log CFU/mL)	NI	NI	NI	NI	NI

NI: not Identified

Besides, the contact angle of apple skin and pear skin to the antimicrobial also contribute to the success of the preservation, where the contact angle of apple skin was 88.9 degrees while pear skin was 8.5 degrees. The apples and pears were evenly covered by 6:20 (w/w). Several references have pointed to the suitability of chitosan for gram-negative bacterial and antifungal[32,14,12,] but with the support of GEO the blending also inhibits the gram-positive *Staphylococcus aureus*. The antimicrobial mechanism of chitosan against bacteria was based on the presence of functional groups of amines that can form bonds with bacterial cell walls and cause intracellular leakage of constituents so that bacteria will cause lysis. Ginger compound constitutes citral (30.8%), zingiberene (17.07%), β-bisabolene Geranyl Acetate (6.7%), 1.8 Cineol (6.1%), geraniol (6.1%).[37] The ketone and aldehyde function groups are active compounds of ginger that function as antifungals. The positively charged amine group makes electrostatic attraction bonds with the H of gingerol and also with the others. Several scientific reports on antifungals in edible found ketones can disrupt fungal cell walls and inhibit the synthesis of polysaccharide 1,3-beta-D-glucan cell wall.[38] Moreover, aldehydes as a group of volatiles can inhibit the fungal cell division and inactivate the functional group involved. Some aldehydes (citral), form a charge-transfer complex in fungal cells to interfere with fungal metabolism.[14] The volatiles with α, β-unsaturated carbonyl groups (such as enones and enables) react with nucleophiles and interrupt the fungal growth.[39]

CONCLUSION

Shrimp shells and ginger are abundant natural resources. The shells were processed to chitosan, which was then mixed with ginger essential oil (GEO). The function of each resource was complimented as blended in the form of films to inhibit bacterias that can delay the ripening of fruits. The task division of chitosan as *Staphylococcus aureus* bacteria gram-positive inhibitor[12] and GEO as gram-negative bacteria inhibitor were successfully in the formula of the mixture chitosan: GEO 6:20 (w/w). It was found to have the highest clear zone radius, which was 10 ± 1.2 mm. This happened to be the optimized formula with lesser weight loss and low colony population viability count applied on apples and pears.

REFERENCES

1. Romanazzi, E. Feliziani, M. Santini, and L. Landi, *Postharvest Biology and Technology*, **75**, 25(2013), https://doi.org/10.1016/j.postharvbio.2012.07.007
2. S. Y. Lee, S. J. Lee, D. S. Choi, and S. J. Hur, *Journal of the Science of Food and Agriculture*, **95(14)**, 2801(2015), https://doi.org/10.1002/jsfa.7218
3. H. Arnon, R. Granit, R. Porat, and E. Poverenov, *Food Chemistry*,**166**, 468(2015),

https://doi.org/10.1016/j.foodchem.2014.06.061
4. K. Kurita, *Marine Biotechnology*, **8(3)**, 220(2006), https://doi.org/10.1007/s10126-005-0097-5
5. M. V. Alvarez, A. G. Ponce, and M. del R. Moreira, *LWT - Food Science and Technology*, **50(1)**, 85(2013), https://doi.org/10.1016/j.lwt.2012.06.021
6. F. Shahidi, J. K. V. Arachchi, and Y. J. Jeon, *Trends in Food Science and Technology*, **10(2)**, 43(1999), https://doi.org/10.1016/S0924-2244(99)00017-5
7. K. W. Kim, R. L. Thomas, C. Lee, and H. J. Park, *Journal of Food Protection*, **66(8)**, 1496(2003), https://doi.org/10.4315/0362-028X-66.8.1495
8. F. E. Firdaus, I. Purnamasari, and P. Gunatama, *MATEC Web of Conferences*, **248**, 3(2018), https://doi.org/10.1051/matecconf/201824804002
9. Y. Pranoto, V. M. Salokhe, and S. K. Rakshit, *Food Research International*, **38(3)**, 268(2005), https://doi.org/10.1016/j.foodres.2004.04.009
10. R. C. Goy, S. T. B. Morais, and O. B. G. Assis, *Brazilian Journal of Pharmacognosy*, **26(1)**, 125(2016), https://doi.org/10.1016/j.bjp.2015.09.010
11. V. Zargar, M. Asghari, and A. Dashti, *ChemBioEng Reviews*, **2(3)**, 212(2015), https://doi.org/10.1002/cben.201400025
12. I. Younes and M. Rinaudo, *Marine Drugs*, **13(3)**, 1155(2015), https://doi.org/10.3390/md13031133
13. Chao Duan, Xin Meng, Jingru Meng, Md. Iqbal Hassan Khan, Lei Dai, Avik Khan, Xingye An, Junhua Zhang, Tanzina Huq, Yonghao Ni, *Journal of Bioresources and Bioproducts*, **4(1)**, 15(2019), https://doi.org/10.21967/jbb.v4i1.189
14. M. Z. Elsabee and E. S. Abdou, *Materials Science and Engineering C*, **33(4)**,1832(2013), https://doi.org/10.1016/j.msec.2013.01.010
15. M. Hosseinnejad and S. M. Jafari, *International Journal of Biological Macromolecules*, **85**, 468(2016), https://doi.org/10.1016/j.ijbiomac.2016.01.022
16. Y. W. Cho, J. Jang, C. R. Park, and S. W. Ko, *Biomacromolecules*, **1(4)**, 611(2000), https://doi.org/10.1021/bm000036j
17. M. C. Cruz-Romero, T. Murphy, M. Morris, E. Cummins, and J. P. Kerry, *Food Control*, **34(2)**, 394(2013), https://doi.org/10.1016/j.foodcont.2013.04.042
18. M. Kumari, H. Mahajan, R. Joshi and M. Gupta, **12**, *Food Packaging and Shelf Life*, 45(2017), https://doi.org/10.1016/j.fpsl.2017.02.003
19. A. Khan, K. D. Vu, B. Riedl, and M. Lacroix, LWT - *Food Science and Technology*, **61(1)**,125(2015), https://doi.org/10.1016/j.lwt.2014.11.035
20. S. Roller and N. Covill, *International Journal of Food Microbiology*, **47(1–2)**, 71(1999), https://doi.org/10.1016/S0168-1605(99)00006-9
21. A. López-Rubio, E. Almenar, P. Hernandez-Muñoz, J. M. Lagarón, R. Catalá, and R. Gavara, *Food Reviews International*, **20(4)**, 367(2004), https://doi.org/10.1081/FRI-200033462
22. B. K. Tiwari, V. P. Valdramidis, C. P. ODonnell, K. Muthukumarappan, P. Bourke, and P. J. Cullen, *Journal of Agricultural and Food Chemistry*, **57(14)**, 5993(2009), https://doi.org/10.1021/jf900668n
23. N. P. Mahalik and A. N. Nambiar, *Trends in Food Science and Technology*, **21(3)**, 123(2010), https://doi.org/10.1016/j.tifs.2009.12.006
24. Y. X. Xu, K. M. Kim, M. A. Hanna, and D. Nag, *Industrial Crops and Products*, **21(2)**,187(2005), https://doi.org/10.1016/j.indcrop.2004.03.002
25. Sawssen Hajji, Islem Younes, Olfa Ghorbel-Bellaaj, Rachid Hajji, Marguerite Rinaudo, Moncef Nasri, Kemel Jellouli, *International Journal of Biological Macromolecules*, **65**, 302(2014), https://doi.org/10.1016/j.ijbiomac.2014.01.045
26. A. Tolaimate, J. Desbrières, M. Rhazi, A. Alagui, M. Vincendon, and P. Vottero, *Polymer*, **41(7)**, 2465(2000), https://doi.org/10.1016/S0032-3861(99)00400-0
27. M. L. Tsaih and R. H. Chen, *Journal of Applied Polymer Science*, **88(13)**, 2919(2003), https://doi.org/10.1002/app.1198
28. T. Okselni, A. Santoni, A. Dharma, and M. *Rasayan Journal of Chemistry*, **12(1)**, 148(2019), https://doi.org/10.31788/RJC.2019.1215019
29. R. R. Surve and S. T. Sankpal, *Rasayan Journal of Chemistry*, **13(1)**, 287(2020),

https://doi.org/10.31788/RJC.2020.1315532
30. R. K. Bai, M. Y. Huang, and Y. Y. Jiang, *Polymer Bulletin*, **20(1)**, 85(1988), https://doi.org/10.1007/BF00262253
31. A. El Ghaouth, R. Ponnampalam, F. Castaigne, and J. Arul, *HortScience*, **27(9)**, 1017(2019), https://doi.org/10.21273/hortsci.27.9.1016
32. I. M. Helander, E. L. Nurmiaho-Lassila, R. Ahvenainen, J. Rhoades, and S. Roller, *International Journal of Food Microbiology*, **71(2–3)**, 239(2001), https://doi.org/10.1016/S0168-1605(01)00609-2
33. H. P. C. A. Cane, N. Saidi, M. Mustanir, D. Darusman, R. Idroes, and M. Musman, *Rasayan Journal of Chemistry*, **13(4)**, 2219(2020), https://doi.org/10.31788/RJC.2020.1345818
34. H. Zengin and A. H. Baysal, *Molecules*, **19(11)**, 17787(2014), https://doi.org/10.3390/molecules191117773
35. Saul Saucedo-Pompa, RomeoRojas-Molina, Antonio F.Aguilera-Carbó, AideSaenz-Galindo, Heliodoro de LaGarza, DianaJasso-Cantú, Cristóbal N.Aguilar, *Food Research International*, **42(4)**, 513(2009), https://doi.org/10.1016/j.foodres.2009.02.017
36. M. Abdollahi, M. Rezaei, and G. Farzi, *International Journal of Food Science and Technology*, **47(4)**, 849(2012), https://doi.org/10.1111/j.1365-2621.2011.02917.x
37. M. Mahboubi, *Clinical Phytoscience*, **5(1)**, 8(2019), https://doi.org/10.1186/s40816-018-0097-4
38. P. M. Sivakumar, T. Muthu Kumar, and M. Doble, *Chemical Biology and Drug Design*, **74(1)**, 72(2009), https://doi.org/10.1111/j.1747-0285.2009.00828.x
39. K. Suresh Babu, Xing-Cong Li, Melissa R. Jacob, Qifeng Zhang, Shabana I. Khan, Daneel Ferreira and Alice M. Clark, *Journal of Medicinal Chemistry*, **49(26)**, 7882(2006), https://doi.org/10.1021/jm061123i

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