Food Research 5 (4) : 266 - 272 (August 2021)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Antimicrobial properties and sensorial acceptability of edible antimicrobial films from seaweed (*Kappaphycus alvarezii)* and cinnamon (*Cinnamomum zeylanicum)* essential oil

[1,*]Siah, W.M., [2]Aminah, A. and [2]Ishak, A.

[1]*Food Science and Technology Research Centre, Malaysian Agricultural Research and Development Institute, Ibu Pejabat MARDI, Persiaran MARDI-UPM, 43400 UPM Serdang, Selangor, Malaysia*
[2]*School of Chemical Sciences and Food Technology, Faculty Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor Darul Ehsan, Malaysia*

Article history:
Received: 3 March 2021
Received in revised form: 28 April 2021
Accepted: 18 June 2021
Available Online: 15 August 2021

Keywords:
Antimicrobial properties,
Edible film,
Essential oils,
Seaweed,
Sensorial acceptability

DOI:
https://doi.org/10.26656/fr.2017.5(4).160

Abstract

Packaging of foods by using edible antimicrobial films (EAF) incorporated with essential oils is able to reduce the spoilage of food due to surface contamination. Essential oils are highly volatile and have strong aromas that can affect their function and consumer acceptance. This paper discussed the effect of cinnamon essential oil (CEO) concentrations on the antimicrobial activity and sensory acceptability of EAF produced from seaweed through a casting method. The CEO was added into the formulations at 10, 20, 30, 40 and 50 g/kg levels. The EAF showed antimicrobial activity against the tested microorganisms with the highest antimicrobial activity at the concentration of 40 g/kg CEO. However, based on the results obtained from a sensory evaluation using a 7-points hedonic scale, the overall acceptability was 3.23 which was lower than the minimum acceptability score of 4. Hence, the highest acceptable level of CEO in the EAF was 30 g/kg. It is recommended that the CEO of 30 g/kg could be incorporated into edible film and act as an alternative method to extend the shelf life of packaged foods.

1. Introduction

Advances in active packaging technologies offer new alternatives for the food industry and consumers to obtain better products and reduce the risk of foodborne illness of packaged food. Active packaging will perform food preservation functions other than the primary roles of providing a barrier to the external environment and protects the foods against deterioration by the actions of microorganisms, moisture and gases (Cooksey, 2010). The packaging will possess antimicrobial activity when antimicrobial agents are added into package materials (Suppakul *et al*., 2003). Slow migration of the antimicrobial agents from the packaging material to the surface of the product localizes the functional effect eventually prevent both contaminations of pathogens and growth of spoilage microorganisms on the food surface (Yildirim *et al*., 2018).

Plant extracts such as essential oils are rich in volatile compounds including terpenoids and phenolic which are highly potential to inhibit a wide spectrum of microorganisms. These active compounds of essential oils will be disturbing the function of the cytoplasmic membrane of microorganisms, disrupting the proton motive force and inhibit the synthesis of protein, thus inhibiting the growth of microorganisms. Some of the most commonly used essential oils in the development of active packaging materials are cinnamon, clove, thyme, turmeric and garlic oils (Cagri *et al*., 2004). The antimicrobial action of different essential oils on different microorganisms was studied and it was recorded that CEO was having a high antimicrobial characteristic (Aitboulahsen *et al*., 2018; Bleoanca *et al*., 2020; Venkatachalam and Lekjing, 2020). Wen *et al*. (2016) reported that pork packed with nanofilm incorporated with CEO decayed after 8 days compared to 3 days for control samples. Meanwhile, Fattahi *et al*. (2020) found that carboxymethyl cellulose edible films added with CEO effectively against the growth of *Aspergillus niger* and *Mucor racemosus.* Aisha and Abdullahi (2017) claimed that bread and salami wrapped with whey protein edible membrane incorporated with CEO maintained fresh after 6 days compared to 3 days for the control samples.

Seaweed is a sustainable natural resource with industrial potential that is not fully utilized in Malaysia.

*Corresponding author.
Email: *wmsiah@mardi.gov.my*

eISSN: 2550-2166 /

Under the Economic Transformation Program (ETP), the Federal Government has approved a substantial amount of fund to uplift the industry to be one of the economic resources for the country and creating more employment opportunities. Hence, a research and development program was needed to support the industry. The development of edible seaweed-based film is a promising method for diversifying the usage and adding value to the seaweed. Although essential oils have many benefits and have been used in food formulations, their use in edible films is still a challenge because their volatility, strong aroma and pungent taste can affect their function, sensory value and consumer acceptance. In an effort to develop new edible and environmentally friendly films for use as active packaging, this study is aimed to evaluate the effect of essential oil concentrations on the antimicrobial activity and sensory acceptability of edible antimicrobial films produced from seaweed and cinnamon essential oil.

2. Materials and methods

2.1 Materials

Cinnamon (*Cinnamomum zeylanicum*) essential oil was obtained from MARDI Kuala Linggi, Malaysia. Dried seaweed (*Kappaphycus alvarezii*) was obtained from Semporna, Sabah Malaysia. *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 11775), *Salmonella enterica* serovar Typhimurium (ATCC 14028), *Aspergillus flavus* (ATCC 26946) and *Saccharomyces cerevisiae* (ATCC 9763) were obtained from Food Science and Technology Research Center MARDI. Medias used were Mueller-Hinton agar MHA (Oxoid CM0337), Tryptone Soya Agar TSA (Oxoid CM0131), Tryptone Soya Broth TSB (Oxoid CM0129), Dekstrosa Sabouraud Agar SDA (Oxoid CM41), Sabouraud Broth SB (Oxoid CM147) and Ringer tablet (Oxoid BR52).

2.2 Films preparation

Edible antimicrobial films (EAF) were prepared following the process in the patented invention titled “Biodegradable Food Film from Seaweed and Process for Producing the Same (MY-173083-A)” and as described in an article by Siah *et al*. (2018). CEO was added into the formulas at 10, 20, 30, 40 and 50 g/kg levels. A film without a CEO served as control. Films were conditioned at 53% relative humidity (RH) and 27±2°C in desiccators over a saturated solution of Mg $(NO_3)2.6H_2O$ for at least 72 hrs according to the Standard Method D618-61 (American Society for Testing and Materials (ASTM), 1993). Films discs of 6 mm diameter were prepared using a puncher. Discs were sterilized with UV light for 5 mins before being tested for their antimicrobial properties.

2.3 Cultures preparation

Pure bacterial colonies were cultured in TSA at 37°C for 24 hrs. For the preparation of bacterial inoculum, 1 colony was taken using a sterile wire loop and transferred into 10 mL of TSB broth and incubated at 37°C for 24 hrs. The broth was then diluted accordingly to 10^6 colony-forming units per mL (CFU/mL) by using a quarter strength of Ringer solution. Pure mould and yeast cultures were grown on the SDA and incubated at 35°C for 48-72 hrs until the formation of colonies. A single colony was taken using a sterile wire loop and transferred to 10 mL of SB broth and incubated at 35°C for 48 hrs. The broth was then diluted accordingly to 10^6 colony-forming units per mL (CFU/mL) by using the quarter strength of Ringer solution.

2.4 Determination of antimicrobial activity of EAF

Testing of the antimicrobial activity of the EAF was carried out using the agar diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS) (1997) with minor modification by Nascimento *et al*. (2000). Mueller-Hinton Agar (MHA) and Dextrose Sabouraud Agar (SDA) were used as a growth medium. For the preparation of media, 38 g of MHA powder or 65 g of SDA powder were added to 1000 mL of distilled water. The mixture was cooked to a boil and sterilized in an autoclave. Sixteen mL of both media were then poured into Petri dishes (as a basal layer) aseptically in the laminar flow chamber and left to turn hard. For the preparation of soft agar (as seeding media) of MHA and SDA, 4 mL of both media were poured into a universal bottle separately and sterilized in an autoclave. After sterilization, soft agars were stored in an oven of 50°C to prevent them from hardening. A total of 0.1 mL of microorganism culture (10^6 CFU/mL) was inoculated into soft agars of MHA and SDA respectively for bacteria and yeast and mould. Well mixed soft agar and respective microorganism culture were poured onto Petri dishes which each contain hardened MHA and SDA. Petri dishes were left for 5 mins in order for the soft agar to turn hard. Each petri dish was divided into 4 segments. Subsequently, 4 sterile EAF discs (6 mm diameter) were transmitted carefully on the surface of an inoculated media by using sterile forceps. Each disc was in the middle of each segment. The film discs without the CEO act as a control. The Petri dishes were incubated at 37°C for 24 hrs for bacteria, and at 35°C for 48 hrs for yeast and mould. Zones of growth inhibition were measured. Only inhibition zones with a diameter greater than 7 mm were considered as positive results. These experiments were carried out in triplicates.

2.5 Sensory evaluation

The sensory evaluation method was according to Aminah (2004). Seaweed films incorporated with 0–50 g/kg CEO were served to the sensory panels to assess the acceptance of the product. A piece of 5×5 cm film from each treatment was served to 30 semi-trained sensory panels. Panels were asked to evaluate all samples based on four sensory attributes (colour, odour, taste and overall acceptability). The scores were calculated using a 7-points hedonic scale, having a range of 1 (dislike extremely), 4 (neither like nor dislike) to 7 (like extremely). Sample attributes that received a score of more than 4 were considered acceptable or favoured by panels.

2.6 Statistical analysis

Data are presented as mean±standard deviation (SD) for at least three separate determinations for each treatment. Experimental data were analyzed by one-way Analysis of Variance (ANOVA) and the significant differences among means were determined by Duncan Multiple Range Test (DMRT) at $p<0.05$ using the Statistical Analysis System (SAS, 2011) computing program.

3. Results and discussion

3.1 Antimicrobial activity of EAF

Results for the antimicrobial effect of films incorporated with different concentrations of CEO to *B. cereus, S. aureus, E. coli, S. enterica* ser. Typhimurium*, A. flavus* and *S. cerevisiae* were showed in Table 1. The antimicrobial activities of these EAF were compared with films without CEO. Results indicated that all the EAF had an antimicrobial activity with a minimal inhibition zone was 8.0 mm and the maximum was 14.33 mm. The control sample without CEO showed no antimicrobial activity indicated that the seaweed and other ingredients used in the formulations of the films do not pose antimicrobial property against the tested microorganisms. When 10 g/kg of CEO was incorporated into the formulation, the film showed antimicrobial activity with the formation of zones of inhibition around the film discs. In general, with the increasing concentrations of CEO in the films, the larger the diameter of the zones of inhibition. A similar trend also observed in an experiment by Frank *et al*. (2018) in their alginate biocomposite films incorporated with CEO where bigger inhibition zones were formed when CEO concentrations increased. Nonsee *et al*. (2011) found similar results with the films made of hydroxypropyl methylcellulose added with clove essential oils. Their results showed that when the concentration of essential oils increased by three folds, the zone of inhibition for *S. aureus, E. coli* O157:H7 and *L. monocytogenes* increased from 28.75 to 39.00, 18.50 to 26.75 and 17.67 to 31.00 mm respectively (diameter of film discs was 17 mm). Ponce *et al*. (2003) reported that the phenolic compounds which are present in the essential oils pose antimicrobial activity. Phenolic compounds at low concentration will impede the enzymes involved in the production of energy; while at the higher concentration will cause protein denaturation.

The EAF with CEO concentrations of 30, 40 and 50 g/kg showed no significant difference ($p>0.05$) in the zone of inhibition for *B. cereus*, *S. aureus* and *E. coli*. When the concentration of CEO in the film reduced to 20 g/kg, there was a significant decrease ($p<0.05$) in the antimicrobial activity, i.e. inhibition zone became smaller for all three types of these microorganisms. Meanwhile, for *S. enterica* ser. Typhimurium*, S. cerevisiae* and *A. flavus*, EAF with 40 and 50 g/kg CEO showed no significant differences ($p>0.05$) in the zone of inhibition. A Similar trend also observed in these three types of microorganisms. The inhibition zone became smaller when the CEO concentrations in the film were reduced. As a conclusion, the maximum inhibition zones for *B. cereus, S. aureus* and *E. coli* were achieved at 30 g/kg CEO, while for *S. enterica* ser. Typhimurium*, S. cerevisiae* and *A. flavus* were at a concentration of 40 g/

Table 1. Effect of *Cinnamomum zeylanicum* concentrations on the formation of inhibition zone

Type of Microorganisms	*Cinnamomum zeylanicum* Concentration (g/kg)					
	0	10	20	30	40	50
B. cereus	0.0±0.0^{d} (-)	8.33±0.58^{c} (+)	10.00±1.08^{b} (+)	12.00±1.00^{a} (+)	12.00±1.00^{a} (+)	12.33±0.58^{a} (+)
S. aureus	0.0±0.0^{c} (-)	9.00±1.00^{b} (+)	10.00±1.00^{b} (+)	12.00±1.00^{a} (+)	12.33 ±0.58^{a} (+)	13.00±1.00^{a} (+)
E. coli	0.0±0.0^{c} (-)	8.00±1.00^{b} (+)	9.00±1.00^{b} (+)	11.00±1.00^{a} (+)	11.33±0.58^{a} (+)	12.00 ±1.00^{a} (+)
S. enterica ser. Typhimurium	0.0±0.0^{d} (-)	8.00±1.00^{c} (+)	8.67±0.58^{bc} (+)	9.67±0.58^{b} (+)	11.00±1.00^{a} (+)	11.33±0.58^{a} (+)
A. flavus	0.0±0.0^{d} (-)	11.00±1.00^{c} (+)	11.33±0.58^{c} (+)	12.67±0.58^{b} (+)	13.67±0.58^{ab} (+)	14.33±0.58^{a} (+)
S. cerevisiae	0.0±0.0^{e} (-)	8.00±1.00^{d} (+)	10.00±1.00^{c} (+)	12.00±1.00^{b} (+)	12.67±0.58^{ab} (+)	13.67±0.58^{a} (+)

Values are expressed as mean±SD of measured diameters of inhibition zone (mm), n = 4. Values with different superscripts in the same row are significantly different at the 5% level ($p<0.05$).
(+) with formation of inhibition zone on the contact surface
(-) without formation of inhibition zone on the contact surface

kg CEO. Thus, the addition of 40 g/kg CEO is sufficient to produce a film with the best antimicrobial effect in this study. Results from other researchers showed that CEO at a concentration of 4 and 5% was effective to inhibit the growth of the studied microorganisms. Aisha and Abdullahi (2017) found their whey protein edible membrane with 4% of CEO exhibited the highest inhibition against *S. aureus*. Sharma *et al*. (2017) reported that 5% CEO in edible biofilm showed most effective against the growth of *E.coli*, *S. aureus* and *L. monocytogenes*.

The EAF with CEO was found to be more effective in inhibiting the growth of Gram-positive bacteria (*B. cereus* and *S. aureus*) compared with Gram-negative bacteria (*S. enterica* ser. Typhimurium and *E. coli*). The structure of the Gram-positive bacteria cell wall allows hydrophobic molecules to easily penetrate the cells and act on both the cell wall and within the cytoplasm. Where else, the cell wall of Gram-negative bacteria is more complex. It has a peptidoglycan layer that is 2-3 nm thick, which is thinner than in the cell wall of Gram-positive bacteria, and composes approximately 20% of the dry weight of the cell. An outer membrane lies outside of the thin peptidoglycan layer. The presence of an outer membrane is one of the features that differentiate Gram-negative from Gram-positive bacteria. It is composed of a double layer of phospholipids that are linked to the inner membrane by lipopolysaccharides and acts as an additional barrier that prevents the lipophilic molecules from penetrating the cell (Nikaido, 2003). Chan *et al*. (2008) explained that antimicrobial compounds in the essential oil are difficult to penetrate the outer membrane of the bacterial cell and thus more resistant. Meanwhile, Gram-positive bacteria only have a layer of peptidoglycan in the cell wall as a barrier layer and easier to penetrate by antimicrobial compounds (Thomas and John, 2006). Similarly, Perry *et al*. (2009) and Ramos *et al*. (2012) also found that Gram-negative bacteria have an additional lipopolysaccharides layer that affected the rate of diffusion of antimicrobial compounds in garlic essential oils and causes active films produced to require higher concentrations to inhibit the growth of Gram-negative than Gram-positive bacteria. The same observations also obtained by Fisher and Phillips (2006), Ojagh *et al*. (2010) as well as Peng and Li (2014). However, Frank *et al*. (2018) obtained opposite results with our finding. They found that their alginate biocomposite films with CEO were more effective against Gram-negative *S. enterica* ser. Typhimurium.

The existence of inhibition zones observed in this study was associated with the main mode of action of phenolic compounds, cinnamaldehyde and eugenol in which they act as a proton exchange causing instability of cytoplasm membrane in the microorganisms and increases its permeability. This condition disrupts the flow of electron and proton motive force and causing adenosine triphosphate discharged and eventually led to the death of microorganisms (Dadalioglu and Evrendilek, 2004; Wenqiang *et al*., 2007; Gómez-Estaca *et al*., 2010). This finding is consistent with the results of Chao *et al*. (2000) who reported that the essential oil of cinnamon completely inhibits the growth of some Gram-negative and positive bacteria, fungi and yeast. Prabha and Ramachandramurty (2013) explained that cinnamaldehyde is an electro-negative compound that disrupts the biological processes that involve the transfer of electrons and reacts with nitrogenous components such as proteins and nucleic acids. This condition causes granulation of the cytoplasm and subsequently, fragmentation of the membrane occurs and inhibits the activity and formation of an important enzyme in the microorganisms. Results from this study indicated the potential use of the CEO as a natural preservative in food packaging technology.

3.2 Sensory acceptability of EAF

Sensory evaluations were conducted on the EAF to determine consumer acceptance of the product. A total of 30 semi-trained panels have evaluated the films and the results are shown in Table 2. The colour of the films plays an important role in influencing consumers' assessment of their acceptance. The formulation without the addition of a CEO produced a clear and transparent film that received a good acceptance from the panels with a score obtained was 6.37. When 10 g/kg CEO was added into the formulation, colour acceptance did not show any significant differences ($p>0.05$) compared with the film without CEO. The results are consistent with the results for the total colour difference (ΔE^*ab) (Siah *et al*., 2018), where these films do not have a significant colour difference whether measured quantitatively by a colourimeter or qualitatively by the sensory panels. The acceptability of the colour attribute decreased significantly ($p<0.05$) when the concentration of CEO in the film exceeding 10 g/kg. The colour score for the film with 10 g/kg CEO was 6.30 and decreased gradually with the increases of CEO concentration and ultimately received a score of 3.67 when 50 g/kg of CEO incorporated into the formulation. The score for the film at 50 g/kg CEO was lower than 4, which is the minimum level of acceptance, thus it can be concluded that film with the CEO concentration at the level of 50 g/kg was rejected by the panels in terms of colour. CEO is naturally yellowish, when it is mixed into the film formulations, the resulting films also become yellowish. When the concentration of CEO increases, the colour

Table 2. Effect of *Cinnamomum zeylanicum* concentrations on sensory score of EAF

Attributes	Concentration of CEO (g/kg)					
	0	10	20	30	40	50
Colour	6.37±0.62[a]	6.30±0.65[a]	5.97±0.67[b]	5.57±0.77[c]	4.53±0.73[d]	3.67±0.61[e]
Odour	6.2±0.710[a]	6.26±0.64[a]	6.15±0.78[a]	5.67±0.74[b]	4.84±0.73[c]	4.17±0.59[d]
Taste	6.03±0.62[a]	5.94±0.71[a]	5.13±0.86[b]	4.50±0.78[c]	3.00±0.53[d]	2.37±0.49[e]
Overall acceptability	6.23±0.63[a]	6.12±0.66[a]	5.50±0.82[b]	4.97±0.72[c]	3.23±0.63[d]	2.53±0.51[e]

Values are expressed as mean±SD, n = 30. Values with different superscripts in the same row are significantly different at the 5% level ($p<0.05$).

intensity of the yellow also increases. Referring to the results obtained for the measurement of whiteness (L*) and yellowness (b*) (Siah *et al*., 2018) by colourimeter, L* values decreased while b* increased when the CEO concentrations increased. Results indicated that the films became less whitish and more yellowish as more CEO incorporated into the film formulations. When there is a decrease in L* and increases in b*, acceptance of the colour attribute in sensory tests also decreased, it is evident that consumers are more tending in a whitish film compared with a yellowish film.

The main volatile organic compound, cinnamaldehyde contributed to the unique and strong odour to the CEO. Consequently, when higher concentrations of CEO were mixed into the film formulations, the resulting films were also stronger in smell. As shown in Table 2, the score for the odour of film without CEO was 6.20 but showed a significant decrease ($p<0.05$) to 5.67 when the concentrations of CEO in the film reaches 30 g/kg. The score continued to decrease significantly ($p<0.05$) in the films with the concentration of 40 and 50 g/kg, respectively 4.84 and 4.17. When the concentration of CEO increases, the smell of cinnamon in the film becomes stronger and this led to the acceptance of the sensory panels to be dwindling. However, up to the concentration of 50 g/kg, the film still got a score of 4.17, exceeded the minimum acceptance threshold of a score of 4. The results show that the films have stronger odour intensity when CEO concentrations increases and sensory panels prefer films that have lower odour intensity compared with the higher ones. This will limit the application of the EAF on the film when the CEO of higher concentrations is used.

The use of CEO in the products must be well controlled as it tastes spicy and the tongue becomes numb if high concentrations are used. The score for taste attribute of films without the addition of CEO was 6.03 as shown in Table 2. The obtained value was a bit lower compared with scores for the colour, odour and overall acceptability of 6.37, 6.20 and 6.23 respectively. For the majority of sensory panels, the edible film is a remarkable product and has had never been encountered before. In addition, the films generally are tasteless and this explains the causes of scores for the taste is comparatively lower. No significant differences ($p> 0.05$) for the score of films with 0 and 10 g/kg CEO. However, when the concentration of CEO was increased to 20 g/kg, the taste score of the films decreased significantly ($p<0.05$) to 5.13. The higher the concentration of CEO in the formulation of the films, the scores were significantly reduced until the concentration of 40 and 50 g/kg, the sensory panels rejected the product in which scores obtained (3.00 and 2.37 respectively) were below the limit of acceptance. All the members of the panel commented that the films with concentrations of 40 or 50 g/kg were too spicy and unacceptable. The results showed that the films had a more pungent intensity when more CEO incorporated. This situation will also limit the applications of the EAF on the film when a higher concentration of CEO is used. The overall acceptability scores of edible films were shown in Table 2. Acceptability of the films with 0 and 10 g/kg CEO had no significant difference ($p> 0.05$) but decreased significantly ($p<0.05$) when the concentration of CEO increased to 20, 30, 40 and 50 g/kg. The level of acceptance by sensory panels was up to 30 g/kg of CEO and films with CEO concentrations beyond 30 g/kg were rejected. The scores for films with CEO at 40 and 50 g/kg were 3.23 and 2.53 respectively, which are below the minimum acceptable level of 4.00. In general, the acceptance of the panel members was most influenced by the taste of the films.

4. Conclusion

Edible films of seaweed incorporated with cinnamon essential oils showed antimicrobial activity against the tested microorganisms using the disc diffusion method. The highest antimicrobial activity was at the concentration of 40 g/kg. However, based on the results obtained from sensory evaluation, the highest acceptable level of CEO in the EAF was 30 g /kg. Thus, it is recommended that the CEO of 30 g/kg could be incorporated into edible film and act as an alternative method to extend the shelf life of packaged foods. Edible films containing plant essential oil provide a new way to enhance microbial safety and shelf life of foods by direct contact with the antimicrobials in the films with the food. The edible seaweed films containing essential oil, therefore, have the potential to provide multiple benefits to the consumers.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This study was supported by the Science Fund Grant (06-03-08-SF0342) from the Ministry of Science, Technology and Innovation, Malaysia (MOSTI).

References

Aisha, I. and Abdullahi, Y. (2017). Development of whey protein concentrate edible membrane with cinnamon essential oil. *Journal of Advances in Biology and Biotechnology*, 11(2), 1-14. https://doi.org/10.9734/JABB/2017/29136

Aitboulahsen, M., Zantar, S., Laglaoui, A., Chairi, H., Arakrak, A., Bakkali, M. and Zerrouk, M.H. (2018). Gelatin-based edible coating combined with *Mentha pulegium* essential oil as bioactive packaging for strawberries. *Journal of Food Quality*, 2018, 8408915. https://doi.org/10.1155/2018/8408915

Aminah, A. (2004). Prinsip Penilaian Sensori. Bangi, Malaysia: Penerbit Universiti Kebangsaan Malaysia. [In Bahasa Malaysia].

American Society for Testing and Materials (ASTM). (1993). Standard practice for conditioning plastics and electrical insulating materials for testing: D618-61. In ASTM Annual Book of American Standard Testing Methods, p. 146-148. Philadelphia, USA: ASTM.

Bleoanca, I., Enachi, E. and Borda, D. (2020). Thyme antimicrobial effect in edible films with high pressure thermally treated whey protein concentrate. *Foods,* 9(7)*,* 855-870. https://doi.org/10.3390/foods9070855

Cagri, A., Ustunol, Z. and Ryser, E.T. (2004). Antimicrobial edible films and coatings. *Journal of Food Protection,* 67(4), 833-848. https://doi.org/10.4315/0362-028X-67.4.833

Chan, E.W.C., Lim, Y.Y., Wong, L.F., Lianto, F.S., Wong, S.K. and Lim, K.K. (2008). Antioxidant and tyrosinase inhibition properties of leaves and rhizomes of ginger species. *Food Chemistry*, 109(3), 477-483. https://doi.org/10.1016/j.foodchem.2008.02.016

Chao, S.C., Young, D.G. and Oberg, C.J. (2000). Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. *Journal of Essential Oil Research*, 12(5), 639-649. https://doi.org/10.1080/10412905.2000.9712177

Cooksey, K. (2010). Active packaging and the shelf life of foods. In Robertson, G.L. (Eds), Food Packaging and Shelf Life, A Practical Guide, p. 373–374. Boca Raton, Florida, USA: CRC Press. https://doi.org/10.1201/9781420078459-c20

Dadalioglu, I. and Evrendilek, G. (2004). Chemical compositions and antibacterial effects of essential oils of Turkish oregano (*Origanum minutiflorum*), bay laurel (*Laurus nobilis*), Spanish lavender (*Lavandula stoechas L.*), and fennel (*Foeniculum vulgare*) on common foodborne pathogens. *Journal of Agricultural and Food Chemistry*, 52(26), 8255-8260. https://doi.org/10.1021/jf049033e

Fattahi, R., Ghanbarzadeh, B., Dehghannya, J., Hosseini, M. and Falcone, P.M. (2020). The effect of Macro and Nano-emulsions of cinnamon essential oil on the properties of edible active films. *Food Science and Nutrition*, 8(12), 6568-6579. https://doi.org/10.1002/fsn3.1946

Fisher, K. and Phillips, C. (2006). The effect of lemon, orange and bergamot essential oils and their components on the survival of *Campylobacter jejuni*, *Escherichia coli* O157, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus* in vitro and in food systems. *Journal of Applied Microbiology*, 101(6), 1232-1240. https://doi.org/10.1111/j.1365-2672.2006.03035.x

Frank, K., Garcia, C.V., Shin, G.H. and Kim, J.T. (2018). Alginate biocomposite films incorporated with cinnamon essential oil nanoemulsions: physical, mechanical, and antibacterial properties. *International Journal of Polymer Science*, 2018, 1519407. https://doi.org/10.1155/2018/1519407

Gómez-Estaca, J., López de Lacey, A., López-Caballero, M.E., Gómez-Guillén, M.C. and Montero, P. (2010). Biodegradable gelatin-chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. *Food Microbiology*, 27(7), 889-896. https://doi.org/10.1016/j.fm.2010.05.012

Nascimento, G.G.F., Locatelli, J., Freitas, P.C. and Silva, G.L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, 31(4), 247-256. https://doi.org/10.1590/S1517-83822000000400003

National Committee for Clinical Laboratory Standards (NCCLS). (1997). Performance Standards for Antimicrobial Disk Susceptibility Test. Ed. 6. Approved Standard. M2-A6. Mayne Island, Canada: NCCLS.

Nonsee, K., Supitchaya, C. and Thawien, W. (2011). Antimicrobial activity and the properties of edible hydroxypropyl methylcellulose based films incorporated with encapsulated clove (*Eugenia caryophyllata Thunb.*) oil. *International Food Research Journal*, 18(4), 1531-1541.

Nikaido, H. (2003). Molecular basis of bacterial outer membrane permeability revisited. *Microbiology and Molecular Biology Review*, 67(4), 593-656. https://doi.org/10.1128/MMBR.67.4.593-656.2003

Ojagh, S.M., Rezaei, M., Razavi, S.H. and Hosseini, S.M.H. (2010). Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. *Food Chemistry*, 120(1), 193-198. https://doi.org/10.1016/j.foodchem.2009.10.006

Peng, Y. and Li, Y. (2014). Combined effects of two kinds of essential oils on physical, mechanical and structural properties of chitosan films. *Food Hydrocolloids,* 36, 287-293. https://doi.org/10.1016/j.foodhyd.2013.10.013

Perry, C.C., Weatherly, M., Beale, T. and Randriamahefa, A. (2009). Atomic force microscopy study of the antimicrobial activity of aqueous garlic versus ampicillin against *Escherichia coli* and *Staphylococcus aureus*. *Journal of the Science of Food and Agriculture,* 89(6), 958-964. https://doi.org/10.1002/jsfa.3538

Ponce, A.G., Fritz, R., Del Valle, C. and Roura, S.I. (2003). Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. *Lebensmittel-wissenschaft Technologie,* 36(7), 679-684. https://doi.org/10.1016/S0023-6438(03)00088-4

Prabha, M.R. and Ramachandramurty, B. (2013). Sequence determination of a novel tripeptide isolated from the young leaves of *Azadirachta indica A. Juss*. *International Journal of Peptides*, 2013, 629549. https://doi.org/10.1155/2013/629549

Ramos, M., Jiménez, A., Peltzer, M. and Garrigós, M.C. (2012). Characterization and antimicrobial activity studies of polypropylene films with carvacrol and thymol for active packaging. *Journal of Food Engineering,* 109(3), 513-519. https://doi.org/10.1016/j.jfoodeng.2011.10.031

SAS. (2011). SAS User's Guide: Statistics Version 9.3. Cary, NC.: SAS Institute Inc.

Sharma, D., Dhanjal, D.S. and Mittal, B. (2017). Development of edible biofilm containing cinnamon to control food-borne pathogen. *Journal of Applied Pharmaceutical Science*, 7(1), 160-164. https://doi.org/10.7324/JAPS.2017.70122

Siah, W.M., Aminah, A. and Ishak, A. (2018). Effect of Cinnamomum zeylanicum essential oil on the physical and mechanical properties of edible films from *Kappaphycus alvarezii*. *Malaysian Applied Biology Journal,* 47(5), 197-203.

Suppakul, P., Miltz, J., Sonneveld, K. and Bigger, S.W. (2003). Active packaging technologies with an emphasis on antimicrobial packaging and its applications. *Journal of Food Science*, 68(2), 408–420. https://doi.org/10.1111/j.1365-2621.2003.tb05687.x

Thomas, F.O. and John, M.S. (2006). Monitoring antimicrobial resistance. In. Amabile-Cuevas, C.F. (Eds). Antimicrobial Resistance in Bacteria, p. 123-148. Norfolk, Australia: Horizon Bioscience.

Venkatachalam, K. and Lekjing, S. (2020). A chitosan-based edible film with clove essential oil and nisin for improving the quality and shelf life of pork patties in cold storage. *Royal Society of Chemistry Advance*, 10(30), 17777-17786. https://doi.org/10.1039/D0RA02986F

Wen, P., Zhu, D.H., Feng, K., Liu, F.J., Lou, W.Y., Li, N., Zong, M.H. and Wu, H. (2016). Fabrication of electrospun polylactic acid nanofilm incorporating cinnamon essential oil/β-cyclodextrin inclusion complex for antimicrobial packaging. *Food Chemistry*, 196, 996–1004. https://doi.org/10.1016/j.foodchem.2015.10.043

Wenqiang, G., Shufen, L., Ruixiang, Y., Shaokun, T. and Can, Q. (2007). Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other traditional extraction methods. *Food Chemistry*, 101(4), 1558-1564. https://doi.org/10.1016/j.foodchem.2006.04.009

Yildirim, S., Rocker, B., Pettersen, M.K., Nilsen-Nygaard, J., Ayhan, Z., Rutkaite, R., Radusin, T., Suminska, P., Marcos, B. and Coma, V. (2018). Active Packaging Applications for Food. *Comprehensive Reviews in Food Science and Food Safety*, 17(1), 165-199. https://doi.org/10.1111/1541-4337.12322

