Analecta Technica Szegedinensia
Vol. 16, No. 01 ISSN 2064-7964 2022

ANTIBACTERIAL EFFECT OF EDIBLE COATINGS WITH ESSENTIAL OIL

Anita Vidács, Balázs Győri, Tamás Lázár*

Department of Food Engineering, Faculty of Engineering, University of Szeged, Mars square 7., 6724, Szeged,
email: vidacsa@mk.u-szeged.hu

ABSTRACT

Food preservation technologies are continuously renewed area because of industrial and customer needs, social transformation, environmentally friendly processing and climate change. The shelf life of perishable food products must be extended with different technologies, for example using green methods like the edible coating (EC). EC is made from different biopolymers (chitosan, alginate, gelatine, agar), the effect can increase using plant extracts. This study examined the effect of chitosan EC, chitosan EC+thyme essential oil (EO); effect of alginate EC, alginate EC+thyme EO on fresh chicken breast having artificial contamination with *Escherichia coli*; *Enterococcus faecalis*, that the EC can extend the shelf life. The organoleptic quality of baked treated chicken breast was also established. Based on the result both EC can decrease the cell number (with 1-3 log CFU/g) on treated chicken breast and this antimicrobial effect was enhanced with thyme essential oil (3.2 µl/ml concentration). There was significant differences ($p<0.05$) between the two edible coatings. Alginate had better preservation effect, than chitosan. However, the thyme EO could increase the antimicrobial activity of chitosan in higher values, than the effect of alginate EC. In this experiment, *E. faecalis* was more sensitive to treatment than *E. coli*. In conclusion, the edible coating can be used as an alternative preservation technique and combined with essential oils can extend the shelf life of chicken breast fillet.

Keywords: chicken breast, edible coating, preservation, thyme essential oil

1. INTRODUCTION

The prevention of food contamination has always been an important point in the food chain. The various food-borne diseases affect not only the food industry but also health and regulatory agencies too. The microbes can contaminate the different food products and one of the most significant source of bacterial contamination is the raw meat. For example, *Listeria* spp., *Escherichia* spp., Salmonella sp., *Staphylococcus* spp., *Pseudomonas* spp., *Enterococcus* spp. are the most common food-borne pathogens/spoilage bacteria [1-4]. There are different processing steps for preservation of food: chilling, freezing, heat processing, canning, drying, smoking, vacuum packaging, modified atmosphere packaging, fermentation, smoking, using different spices, adding preservatives and use other new technology (active packaging, high hydrostatic pressure, irradiation, edible coating) [5-6].

The edible coatings can be a potential approach to extend the shelf life of meat and these are bio-based packaging materials [7]. The edible coating is a thin layer of edible material, which coats the food, usually used in liquid form and with immersion method on food [8]. Chitosan is a polysaccharide, derived from chitin, nontoxic, biodegradable, having antimicrobial and film-forming properties. Chitosan film has selective permeability to oxygen and carbon dioxide. [5, 7, 9-12] Sodium-alginate is derived from seaweed, algae, or synthetized by microorganisms used as gel-forming and colloidal stabilizing agents in the food industry. Alginate solution can form gel with different ions (calcium, magnesium, iron, aluminium), gel is water-soluble, flexible, tasteless, odorless, low permeable to oxygen and oils. [13-15]

Plant extracts, including essential oils (EOs), show antibacterial and antifungal effect, suggesting that they can become novel antimicrobial compounds. Most of the EOs are generally recognized as safe (GRAS). [16-17]

Therefore, this study examined the shelf-life extension ability of chitosan and alginate edible coatings on chicken breast fillet having *Escherichia coli* and *Enterococcus faecalis* contamination. Beside this, the preservation effect of coating was enhanced with thyme essential oils.

DOI: https://doi.org/10.14232/analecta.2022.1.71-76

2. MATERIALS AND METHODS

2.1. Bacterial strains

One Gram-positive bacterium (*Enterococcus faecalis*) and one Gram-negative bacterium (*Escherichia coli*) were used during the research. For culturing and for determination of minimum bactericidal concentration, TGE (Tryptone-Glucose-Extract) broth and agar was used (10 g glucose - VWR, Hungary; 5 g tryptone - VWR, Hungary; 2.5 g yeast-extract – Merck, Hungary; 20 g agar-agar - VWR, Hungary). The incubation temperature was 37 °C.

2.2. Minimum bactericide concentration (MBC)

For determination of MBC values on bacteria, the thyme essential oil (tEO) (Aromax Natural Products Zrt., Hungary) was investigated with macrodilution method in different concentrations (in the range of 50 to 0.1 mg/ml). Tween 40 was used to disperse the EOs in the medium. The used cell suspension was 24-h old and the cell number was 10^6 CFU/ml. In previous tests 1% Tween 40 did not affect the growth of the investigated bacteria [18]. The inoculated broth without EO served as control. After 24 hours incubation at 37 °C MBCs were determined by the tracking plate method [19] transferring 10 µl from the microplate wells to TGE agar for the enumeration of living cells. After 24-h incubation at 37 °C, the number of colonies was counted. MBC was defined as the EO concentration where no colony growth was observed on TGE agar.

2.3. Coating preparation

Chitosan (CH) edible coatings: 2 % (wt/v) Chitosan (Sigma-Aldrich) was added to distillated water and acidified with 1 % acetic acid (v/v) (Sigma-Aldrich).
Alginate (AL) edible coatings: 1.5 % (wt/v) Sodium-alginate (Sigma-Aldrich) and 1.5 % (wt/v) $CaCl_2$ (wt/v) was used for the coating preparation. The food sample was immersed firstly in the alginate solution and after that in $CaCl_2$ liquid for crosslinking the alginate (egg-box model/gelation mechanism [20]).
The effect of edible coating was enhanced with tEO in MBC concentration. The tEO was mixed with chitosan, alginate solution (25 °C).

2.3. Antibacterial effect of edible coatings on chicken breast fillet

The chicken breast fillet was obtained from Spar market. The 10-10 g of fillet were prepared for the coatings: each of 10 g fillet was immersed in *E. coli* suspension (10^6 CFU/ml, 10 min) for homogenous dispersion of bacterium on surfaces. After that, the fillets were divided three parts: control-without edible coating; edible coating; edible coating +tEO. The control was placed in sterile plastic box. Second part of the contaminated sample was immersed in 2% CH solution for 10 minutes and place in sterile plastic box; the third part of fillet was immersed in 2% CH solution having tEO in MBC for 10 minutes and place in sterile plastic box. The experiments of alginate coatings were taken at the same way with having one extra step, after the immersion in 1.5 % AL solution/AL+tEO the sample was put in 1.5% $CaCl_2$ solution for 10 minutes. Control and the treated samples in triplicates were stored at 8°C in plastic box and the cell numbers were monitored at 0, 24, 120, 168 hours. 10-10 g of fillet sample were mashed up in 90 ml steril water, prepared 10-fold serial dilutions and surfaced 0.1 ml onto TGE agar and incubated 37°C and for 24 h. After the incubation the remained cell number was established with colony counting.
The study of *E. faecalis* was designed as *E. coli* experiments.

The statistical differences was established by using T-test, Microsoft Excel software.

DOI: https://doi.org/10.14232/analecta.2022.1.71-76

The sensory evaluation was made with scoring method (points were awarded from 1 to 5 point: 5 excellence; 1 not accepted), where the panelists were 12 non-trained people. The organoleptic properties were the taste, odour, texture and the acceptance. The results were the average points of each of properties. The chicken fillets were covered with edible coatings having thyme essential oils and baked in pan.

3. RESULTS AND DISCUSSION

3.1. Minimum bactericide concentration of thyme essential oil

The MBC was established where were not colony formation on the surfaces of agar. Both bacteria had 3.2 mg/ml MBC values, based on this study there was not difference in sensitivity against tEO between the *E. coli* and *E. faecalis*. In the further experiments, 3.2 mg/ml tEO was used.

3.2. Effect of coatings on chicken breast contaminated with E. coli

Based on the results, the chitosan and alginate coating had an inhibitory/antibacterial effect; the cell number was reduced with 0.5-3 log CFU/g on chicken breast fillet (Diagram 1.). The control cell number was increased from 5.66 log CFU/g (24 h: 7.26 log CFU/g; 120 h: 9.10 log CFU/g) to 10.01 log CFU/g for the 168 hours. Despite of that, the AL treated sample had a slow rising: 5.49 log CFU/g, 6.46 and 6.86 log CFU/g, respectively 24, 120 and 168 h; the coating could inhibit the growing of the bacterium. There was the same tendency by the CH, but the cell number was higher, 6.60, 6.91, 7.25 log CFU/g. The preservation effect was enhanced with added tEO, mainly the effect of CH was improved. After 24 hours, the EO could help in the preservation (there was a bactericide effect with the tEO), because there was significant difference between the edible coating and edible coating+ tEO treatment ($p<0.05$). At the 120-168h experiment, there was not differences between the treatments, perhaps the volatile components of EO had not effect on the bacterium and the tEO could only supported the inhibitory effect of CH coating. Sogut and Seydim [21] observed that, the edible chitosan film could decline the *E. coli* cell number by 1.0-1.5 log CFU/g in vitro. In other research, the cinnamon essential oil could improve the antibacterial effect of CH edible coating [10].

DOI: https://doi.org/10.14232/analecta.2022.1.71-76

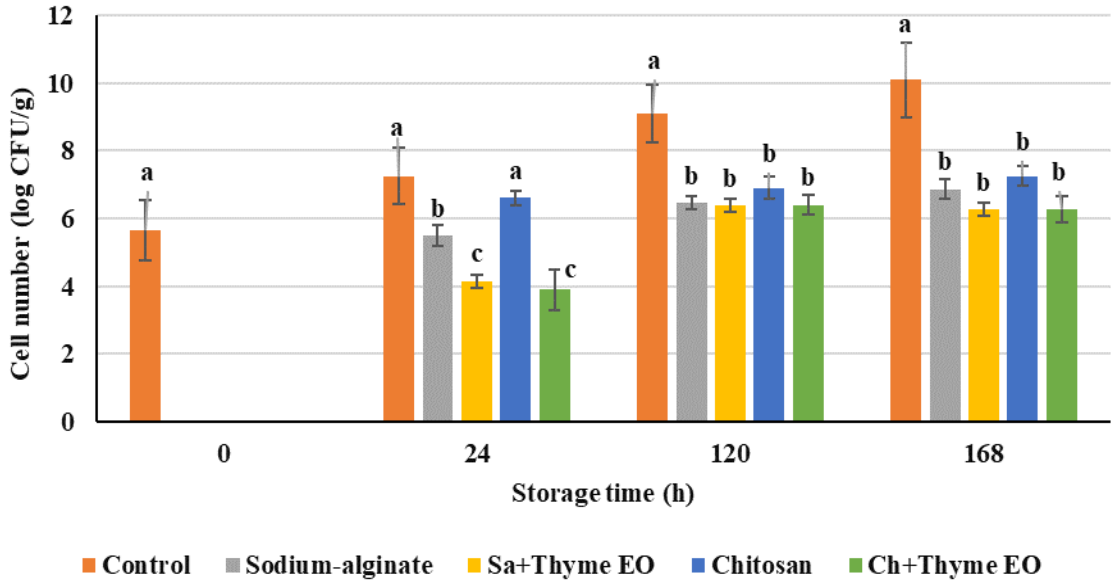

Diagram 1. Effect of edible coatings on E. coli (Different small letters indicate significant differences (p< 0.05))

The chitosan and alginate edible coating had a preservative effect on *E. faecalis* contaminated chicken breast fillet (Diagram 2.). There was same results as on *E. coli*, the AL could inhibit better cells in the first 24 hours, than CH. However, there were no significant differences between the CH and CH+tEO treatment, but the thyme EO raised the effect of AL edible coating, at the end, there was declining by 5 log CFU/g in the cell number. The *E. faecalis* was more sensitive to tEO in edible coating, than *E. coli* (Diagram 1-2.).

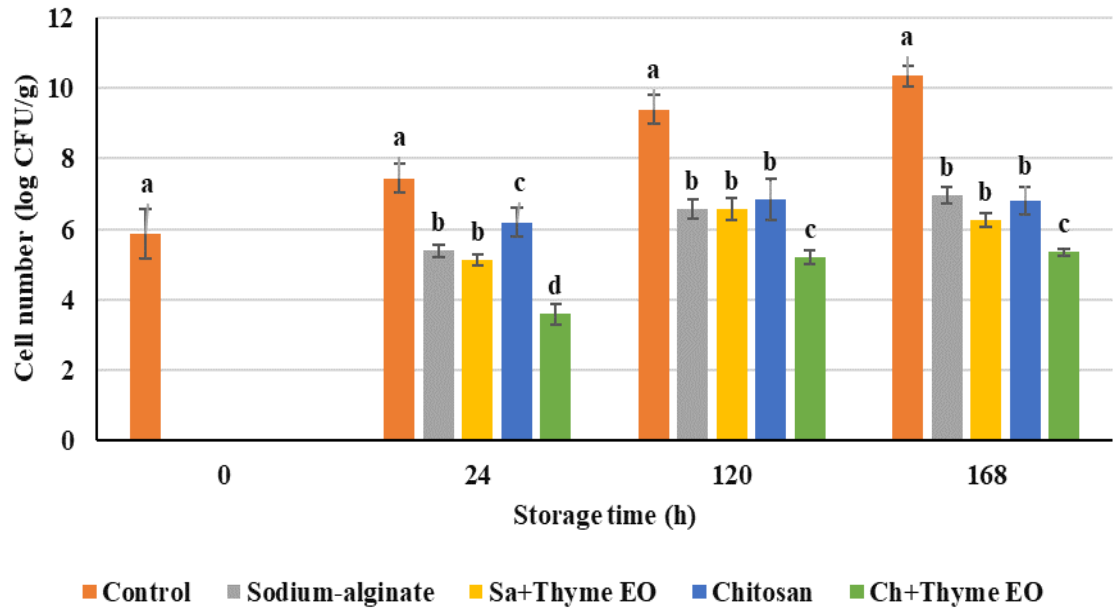

Diagram 2. Effect of edible coatings on E. faecalis (Different small letters indicate significant differences (p< 0.05))

DOI: https://doi.org/10.14232/analecta.2022.1.71-76

At the sensory evaluation, the taste of the prepared chicken fillet was around 3, and the chitosan+tEO got better points. In contrast, the odour of chitosan+tEO was scored down (2.3). In the background, perhaps, the gas permeability and water holding capacity of the two ECs were different, for this needs more examination. The overall acceptance was same, the panelists added 4.1-4.3 point (maximum point was 5).

Based on the results, the used edible coatings had a preservative effect on chicken breast fillet among 1 week, it could be an alternative way for extended the shelf life of raw meat. There is different sensitivity between the bacteria, there need more research for establishment the difference between the microbes, and how could be standardized the using of edible coating for preservation. Attention should also be attended to improving sensory properties in the future. It will be found that extracts, which match the best to the particular food.

4. CONCLUSIONS

Given today's global challenges (sustainability, environmentally friendly, renewable technology, customer demands), the need for new, green, alternative methods/technology for prolonging the shelf life of food with preserving or enhancing its impact on health is increasingly intensive. These biopolymer edible coatings in alone and combined with thyme EO were found to be effective against two investigated bacteria, can prolong the shelf life in refrigerated storage. Future studies are needed to establish the shelf-life extension effect of edible coatings on more different food, which have contaminated other microbes or multiple-species.

REFERENCES

[1] M. S. Fernandes, D. Y. Kabuki, A.Y. Kuaye. Behavior of Listeria Monocytogenes in a Multi-Species Biofilm with Enterococcus Faecalis and Enterococcus Feacium and Control Through Sanitation Procedures. Journal of Food Microbiology, 200 (2015), pp. 5-12. https://doi.org/10.1016/j.ijfoodmicro.2015.01.003

[2] E. Giaouris, E. Heir, M. Hébraud, N. Chorianopoulos, S. Langsrud, T. Møretrø, O. Habimana, M. Desvaux, S. Renier, G. J. Nychas, G. J. Attachment and Biofilm Formation by Foodborne Bacteria in Meat Processing Environments: Causes, Implications, Role of Bacterial Interactions and Control by Alternative Novel Methods. Meat Science, 97 (3) (2014), pp. 298-309. https://doi.org/10.1016/j.meatsci.2013.05.023

[3] O. Rendueles, J.M. Ghigo. Multi-Species Biofilms: How to Avoid Unfriendly Neighbors. FEMS Microbiology Reviews, 36 (2012), pp. 972-989. https://doi.org/10.1111/j.1574-6976.2012.00328.x

[4] M. Simões, L. C. Simões, M. J. Vieira. A Review of Current and Emergent Biofilm Control Strategies. LWT-Food Science and Technology, 43 (2010), pp. 573-583. https://doi.org/10.1016/j.lwt.2009.12.008

[5] B. Schumann, M. Schmid. Packaging Concepts for Fresh and Processed Meat – Recent Progresses. Innovative Food Science and Emerging Technologies, 47 (2018). https://doi.org/10.1016/j.ifset.2018.02.005

[6] G. H. Zhou, X. L. Xu, Y. Liu. Preservation Technologies for Fresh Meat – A Review. Meat Science, 86 (2010), pp. 119–128. https://doi.org/10.1016/j.meatsci.2010.04.033

[7] L. Motelica, D. Ficai, A. Ficai, O. Oprea, D. Kaya, E. Andronescu. Biodegradable Antimicrobial Food Packaging: Trends and Perspectives. Foods, 9 (2020), pp. 1438. https://doi.org/10.3390/foods9101438

[8] V. Falguera, J. P. Quintero, A. Jiménez, J. A. Muñoz, A. Ibarz. Edible Films and Coatings: Structures, Active Functions and Trends in Their Use. Trends in Food Science and Technology, 22 (6) (2011), pp. 292-303. https://doi.org/10.1016/j.tifs.2011.02.004

DOI: https://doi.org/10.14232/analecta.2022.1.71-76

[9] V. Coma, A. Martial-Gros, S. Garreau, A. Copinet, F. Salin, A. Deschamps. Edible Antimicrobial Films Based on Chitosan Matrix. Journal of Food Science, 67 (2002), pp. 1162-1169. https://doi.org/10.1111/j.1365-2621.2002.tb09470.x
[10] M. Z. Elsabee, E. S. Abdou. Chitosan Based Edible Films and Coatings: A Review. Materials Science and Engineering: C Biomaterials, 33 (4) (2013), pp. 1819-41. https://doi.org/10.1016/j.msec.2013.01.010
[11] D. H. Ngo, T. S. Vo, D. N. Ngo, K. H. Kang, J. Y. Je, H. N. D. Pham, H. G. Byun, S. K. Kim. Biological effects of chitosan and its derivatives. Food Hydrocolloids, 51 (2015), pp. 200-216. https://doi.org/10.1016/j.foodhyd.2015.05.023
[12] R. Ribeiro-Santos, M. Andrade, N. R. de Melo, A. Sanches-Silva. Use of Essential Oils in Active Food Packaging: Recent Advances and Future Trends. Trends in Food Science and Technology, 61 (2017), pp. 132-140. https://doi.org/10.1016/j.tifs.2016.11.021
[13] D. S. Cha, M. S. Chinnan. Biopolymer-Based Antimicrobial Packaging: A Review. Critical Reviews in Food Science and Nutrition, 44 (4) (2004), pp. 223-237. https://doi.org/10.1080/10408690490464276
[14] A. Kurucz, E. Gyimes. Edible Film Coatings for the Packaging of Pre-Cooked Poultry Meat Products (frankfurters). Journal of Food Investigation 62 (2016), pp. 952-955.
[15] E. Tavassoli-Kafrani, H. Shekarchizadeh, M. Masoudpour-Behabadi. Development of Edible Films and Coatings from Alginates and Carrageenans. Carbohydrate Polymers, 137 (2016), pp. 360-374. https://doi.org/10.1016/j.carbpol.2015.10.074
[16] S. Burt. Essential Oils: Their Antibacterial Properties and Potential Applications in Foods - A Review. International Journal of Food Microbiology, 94 (2004), pp. 223–253. https://doi.org/10.1016/j.ijfoodmicro.2004.03.022
[17] FDA.gov, Food Additive Status List [online] Available at:https://www.fda.gov/food/food-additives-petitions/food-additive-status-list#additives (Accessed 21.02.2022)
[18] E. B. Kerekes, A. Vidács, M. Takó, T. Petkovits, C. Vágvölgyi, G. Horváth, V. L. Balázs, J. Krisch. Anti-Biofilm Effect of Selected Essential Oils and Main Components on Mono- and Polymicrobic Bacterial Cultures. Microorganisms, 7 (2019), pp. 345. https://doi.org/10.3390/microorganisms7090345
[19] B. D. Jett, K. L. Hatter, M. M. Huycke, M. S. Gilmore. (1997): Simplified Agar Plate Method For Quantifying Viable Bacteria. BioTechniques, 23 (1997), pp. 648-650. https://doi.org/10.2144/97234bm22
[20] L. Cao, W. Lu, A. Mata, K. Nishinari, Y. Fang. Egg-Box Model-Based Gelation of Alginate and Pectin: A Review. Carbohydrate Polymers, 242 (2020), pp. 116389. https://doi.org/10.1016/j.carbpol.2020.116389
[21] E. Sogut, A. C. Seydim. The Effects of Chitosan and Grape Seed Extract-Based Edible Films on the Quality of Vacuum Packaged Chicken Breast Fillets. Food Packaging and Shelf Life, 18 (2018), pp. 13-20. https://doi.org/10.1016/j.fpsl.2018.07.006

DOI: https://doi.org/10.14232/analecta.2022.1.71-76