ChemTech

International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.4, pp 233-244, 2017

Antimicrobial Edible Film Based Whey Protein – A Review

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Abstract : Some perishable food need a edible packaging materials to protect it from deterioration, edible film whey protein containing antimicrobial compound is one of the most interest as packaging material. Whey protein edible film containing organic acid (propionic, benzoic, sorbic and lactic acid), enzyme (lysozyme) and liquid smoke showed an inhibition ability the growth some bacteria, yeast and mold in food which coated using whey protein edible film. Antimicrobial activity of whey protein edible film containing antimicrobial compound can prolong its inhibition activity if the antimicrobial compound attached in some substance such as beeswax, oleic acid and polyacrilic acid to controlling the release of antimicrobial compound from whey protein edible film.

Introduction

Foods are generally susceptible to microbiological, physical and chemical deterioration during storage and distribution, both as a function of food composition and the environmental conditions[1]. A selection of packaging materials can prevent food deterioration by providing barrier[2], using the materials wich biodegradable, non-toxic and biocompatible[3,4]. Edible film based biodegradable substance showed antimicrobial activity[5].

Food packaging is widely used for quality protection, to extending the shelf life of perishable food and ensuring food hygiene[6]. Edible films is an environmentally-friend and have ability to protect perishable food from deterioration by reducing microbial growth. Several research reported on the efficacy of films containing antimicrobials agent to control microbial growth on food, so edible films is considered as alternative method to extend the quality of food[7,8,9,10].

Edible film provide additional protection against contamination of microorganism and modified atmosphere of food[11]. Food packaging become considerable attention attributed with natural polymers development as eco-friendly packaging materials. Such natural polymers may be lipid, polysaccharide or protein based could be used as edible films formula[12].

Antimicrobial whey protein films.

Whey proteins are a renewable source and a by-product of cheesemaking industry[13], it have been employed as biodegradable packaging material[14] . Whey protein isolates (WPI) are the purer form of whey proteins[15]. WPI is isolated from a byproduct of cheese manufacturing, which makes bio-based packaging materials production (Ozer et al., 2016). WPI have excellent film forming[14], produce transparent films and provide certain mechanical properties[16,17], moderate moisture permeability[18], and good gas barrier properties—comparable to high density polyethylene, low-density polyethylene (LDPE), vinyl alcohol, ethylene vinyl alcohol, polyvinylidene chloride (PVDC) and polyester[19,20].

The ability of WPI edible films as barriers to solute and gas will enhance food shelf-life and quality[21]. However, these films have lower mechanical and higher water vapor permeability barrier because their hydrophilic properties[21]. The hydrophilic properties of WPI films causes active compound quickly release from the film when contact with water. The release of active compounds from WPI films mainly depends on hydrophilic properties of WPI and active compound[22].

WPI films are good vehicles to incorporate antimicrobial compound, for example essential oils, bacteriocins such as nisin[23] and natamycin [24], plant extracts such as essential oils[25] and viable cells of certain microorganisms. Nowadays, the development active package containing natural antimicrobials, increase consumer demand for natural food additives.

Edible films can serve as carriers for antimicrobial compound that can reduce pathogenic and food spoilage organisms growth on food surfaces. Some antimicrobial compound commonly used as preservatives[26,27,28,29,30,31,32] are organic acids (e.g. acetic, citric, lactic and propionic acids), bacteriocins (e.g. nisin), enzymes (e.g. lysozyme), polysaccharides (e.g. chitosan), and several plant extracts[33,34,35].

WPI films are excellent carriers of food additives as antimicrobials and spices to improve the functionality of the packaging based WPI[36, 37].

The strategy for controlled release of antimicrobial compound from whey protein films.

The release of antimicrobial compound from hydrophilic edible film such as whey protein film while maintaining antimicrobial efficacy of the films over time is still a challenging issue. This is a large problem in the films made from hydrophilic polymers, because the swelling of polymer such as whey protein in the presence of water. The swelling of polymer increase free volume between polymer chains, in turn accelerating antimicrobial compound release. Considering some limitations of approaches, several strategy developed for sustained antimicrobial compound release from whey protein film[14] as shown at Table 1..

Polyacrylic acid (PAA)

Encapsulated lysozyme incorporated into a food-grade polyelectrolyte, polyacrylic acid (PAA) a weak polyelectrolyte, to form lysozyme/PAA complexes. The complexes is mostly driven by oppositely charged groups in the protein and the polyelectrolyte via electrostatic interactions[38,39,40,41]. The complex is formed spontaneously under certain conditions by mixing proteins and polyelectrolytes, subsequently the complex incorporated to whey protein isolate films[14]. Lysozyme-polyacrylic acid complex can be used to achieve whey protein isolate film with long-lasting antimicrobial effect and whey protein isolate films prepared with lysozyme-polyacrylic acid complex suitable as food packaging materials[14].

Lysozyme- polyacrylic acid complex incorporated into whey protein isolate films was effective to control the release rate of lysozyme from the film, allowing sustained lysozyme release for extended periods. The films prepared using Lysozyme- polyacrylic acid complex have a great potential for food shelf-life extension and food preservation[14].

Beeswax

The whey protein films with beeswax have antimicrobial activity against Listeria innocua[42]. Whey protein film containing lysozyme using beeswax as hydrophobic compound of film increased the total of lysozyme released from whey protein film. Antimicrobial film based whey protein can improve food safety of food stored in the refrigerators[42]. . Beeswax in whey protein film containing organic acid decrease the release of organic acids from whey protein film and the film showed antimicrobial avtivity against Escherichia coli and Salmonella spp. [43].

Oleic acid

Whey protein-oleic acid film containing lysozyme showed inhibition of L. innocua, antimicrobial films of Whey protein-oleic acid could be activated to improve food safety[42].

Antimicrobial activity of whey protein edible film containing antimicrobial compound

Edible film containing antimicrobial compoud has been approved have antimicrobial ability against several microorganism to extend the shelf life of food using several antimicrobial compound includes organic acids, enzyme, cell free supernatan and liquid smoke.

Lysozyme

Hen egg white lysozyme is the most potential candidates for antimicrobial compound incorporating into whey protein film, because its good stability and activity in edible films and food systems during storage in refrigerated temperatures[44,45]. Lysozyme is enzyme that found in hen, plants, mammals and bacteria, this enzyme is used as food preservative[46]. It have antimicrobial effect on Gram positive bacteria via peptidoglycan destroying especially to hydrolyze beta-glycosidic bonding between N-acetylmuramic and N-acetyl Glucosamine[47], acetylglucosamine[48,49], in the peptidoglycan of bacterial cell wall. It is frequently used in the antimicrobial edible film[50].

The antimicrobial activity of lysozyme mainly on Gram-positive by destroying the peptidoglycan in cell walls bacteria especially by splitting the bonds between N-acetylmuramic acid and N-acetylglucosamine[48,49]. The application of lysozyme in film mainly the inhibition of Gram-positive. *Listeria monocytogenes* a Gram-positive pathogenic bacteria is inhibited by lysozyme to prevent bacterium infections[51].

Cell-free supernatant of *Lactobacillus sakei*

Cell-free supernatants from probiotics bacteria contain some antimicrobial compound (such as bacteriocins, lactic acid and acetic acid) thus potential to incorporate into edible film[52]. *Lactobacillus sakei* is a lactic acid bacteria using as biopreservatives have been added to meat products, dur to their ability to inhibit *E. coli* O157:H7[53]. WPI films incorporated with cell-free supernatant of *Lb. sakei* have ability to inhibit *L. monocytogenes* and E. coli growth during refrigerated storage[54].

Potassium sorbate

Potassium sorbate can effectively restrain the activity of yeast, mould and aerophile bacteria. Potassium sorbate ionizes to form sorbic acid when dissolved in water. Potassium sorbate is a weak acid and increased the ability to penetrate into cytoplasmic membrane of bacteria especially in the undissociated form[55].

The addition of potassium sorbate in whey protein concentrate films led to inhibit the growth of Shiga toxin-producing *Escherichia coli* (STEC) O157[56]. Potassium sorbate was incorporated into whey protein concentrate/glycerol edible films shows inhibition of the growth of Shiga toxin producing *Escherichia coli* (STEC). STEC growth inhibition was dependent on potassium sorbate content. The active packaging based on whey proteins and potassium sorbate to prevent STEC growth may be an effective and safe alternative to be used as active packaging in food industry[57].

Sorbic acid

Incorporation for sorbic acid or or p-aminobenzoic acid into whey protein isolate films (pH 5.2) can inhibit *Listeria monocytogenes, S. typhimurium and Escherichia coli* O157:H7 growth[58]. Sorbic acid is used as preservative agents, due to its safety and low influence on organoleptic properties[59]. Sorbic acid is classified as GRAS additive and effective toward yeast and moulds but only partly retard bacterial growth [60,61]. This antimicrobial compound has been used in antimicrobial films and effective in controlling the growth of microorganism[62], such as *Escherichia coli, Penicillium spp* and *Lactobacillus plantarum*[63].

Benzoic acid

Benzoic acid incorporated into whey protein film has been reported on the antimicrobial activity against *Escherichia coli* and *Salmonella spp.* [64].

Lactic acid

Whey protein film containing lactic acid has been reported on the antimicrobial activity against *E. coli* and *Salmonella spp.* [64]. Edible film containing lactic acid and chitooligosaccharide showed the effect against Gram-negative bacteria (*Escherichia coli, Pseudomonas fluorescens, Salmonella spp.*) and Gram-positive bacteria (*Listeria innocua, Staphylococcus aureus*) and yeast (*Yarrowi lipolytica*) [13]. Adding lactic acid to foods for preservation purposes, via elimination of spoilage and pathogenic bacteria growth[65]. However, lactic acid may not exhibit a significant antimicrobial activity toward yeasts and molds [66].

Propionic acid

Incorporating propionic acid in whey protein film showed antimicrobial activity against *E. coli* and *Salmonella spp.* [64]. Propionic acid has antifungal performance and capable to inhibit the growth of Gram-negative and Gram-positive bacteria. Propionic acid is usually applied to inhibit mold growth on cheese and butter, as well as to hamper bacteria and yeasts growth in syrup[67].

Liquid smoke

Liquid smoke is natural wood smoke flavors solution manufactured by condensing wood smoke[68]. Liquid smoke was offer many advantages than traditional smoking, namely speed of application, easy and product uniformity. Commercial liquid smoke products contain phenols, acetic acid and carbonyl compounds, which are shown bactericidal effect at relatively low content. Liquid smoke can inactivate food spoilage organisms and food-borne pathogens, including *Salmonella sp., Staphylococcus aureus, Escherichia coli,* and *Listeria monocytogenes*[67,68,69,70,71,72,73,74]. Based on these evidences, makes liquid smoke a potentially attractive as antimicrobial compound in antimicrobial edible films [75]. The incorporation of commercial liquid smoke to whey protein concentrate-based edible films useful to prevent the growth of foodborne pathogen such as *Listeria monocytogenes*[76].

Antimicrobial activity of whey protein edible film containing antimicrobial compound

Edible film containing antimicrobial compoud has been approved to extend the shelf life and food safety of food against against several microorganism includes bacteria, yeast and mold as shown at Table 1.

Table 1. Antimicrobial whey protein film

Treatment	Antimicrobial agent	Effect/result	Microorganism	Reference
Whey protein isolate (WPI) films supplemented with *Lb. sakei* cell-free supernatan performed on wrapped beef.	Cell-free supernatant of *Lactobacillus sakei* NRRL B-1917	WPI films supplemented with *Lb. sakei* cell-free supernatant (containing a bacteriocin-like substance) exhibited antimicrobial activity against *L. monocytogenes* and *E. coli*, and maintained antimicrobial activity on beef inoculated with *L. monocytogenes* or *E. coli*.	*E. coli* and *L. monocytogenes*	75
Incorporating organic acids in whey protein film	acetic, lactic, propionic and benzoic acids	incorporating organic acids in whey protein film showed antimicrobial activity against *Eschericia coli* and *Salmonella sp*.	*Eschericia coli* and *Salmonella sp.*	62
The release of lysozyme from whey protein film containing	Lysozyme	Incorporating of beeswax decreasing the release of lysozyme from whey protein film, and stable antibacterial	*E. coli*	43

beeswax		activity against *E. coli*		
Sodium cyanoborohydride addition on the release of lysozyme from whey protein film	Lysozyme	Increasing sodium cyanoborohydride content decreasing the release of lysozyme from whey protein film, and stable antibacterial activity against *E. coli*	*E. coli*	77
Whey protein film containing lysozyme as gouda cheese edible coating	Lysozyme	Antimicrobial composite edible film containing lysozyme effective to inhibit microorganism growth both at the surface and inside region of gouda cheese during ripening.	Lactic acid bacteria, *Enterococcus, Coliform, E. coli, Salmonella, S. aureus* and yeast/mold).	76
Controlling the release of lysozyme from WPI in smoked salmon	Hen egg white Lysozyme	Antimicrobial packaging using lysozyme, oleic acid and whey protein films showed inhibition of *L. innocua* growth on coated salmon slices stored at 4°C.	*L. innocua*	42
Incorporated polyacrylic acid lysozyme complexed on WPI film.	Lysozyme	Complexation of lysozyme-polyacrylic acid was an effective tool for controlling lysozyme release from WPI films. Complexation reduced the release rate of lysozyme from WPI films.	*Listeria innocula*	14
Incorporated pottasium sorbate into WPC films toretard non-O157 STEC	Potassium sorbate	The addition potassium sorbate in WPC films led to retard the growth of STEC pathogens, Antimicrobial edible films containing most organic acids frequently used as preservatives are more effective in an acidic environment.	*E. coli* O157:H7 ATCC 43895, Eight non-O157 Shiga toxin-producing *E. coli*	55
Potassium sorbate included into WPC films to retard Shiga toxin-producing *E. coli*	Potassium sorbate	WPC film containing potassium sorbate retarded the growth of Shiga toxin-producing *E. coli* may be an ecological, effective, safe and relatively cheap alternative to be food packaging industry.	Shiga toxin-producing *E. coli*	30
Inhibition of spoilage microflora in cheese using antimicrobial WPI film	Chitooligosaccharide, lactic acid, sodium benzoate, ctric acid. nisin	Lactic acid and chitooligosaccharide (COS) exhibited bacteriocidal effect on Gram-positive and Gram-negative bacteria, respectively, whereas sodium benzoate and COS exhibited the highest inhibitory effect against the yeast. Combinations of lactic acid	*Escherichia coli, Listeria innocua, Yarrowia lipolytica, Staphylococcus spp., Pseudomonas spp., Enterobacteriacea,* yeasts and moulds	80

		and COS showed the highest effect to all microorganisms tested; The application on the cheese surface, they more active against bacteria, and less effective toward yeasts and moulds than their commercial coating counterparts.		
Incorporation several organic acid on . from WPI film.	lactic acid (LA) and propionic acid (PRO), chitooligosaccharides (COS) and natamycin (NA)	WPI Films formulated with LA, PRO or COS showed antimicrobial activity toward all microorganisms tested. COS most active against Gram-negative bacteria, whereas LA was the most active toward Gram-positive ones. NA was not active toward bacteria, but showed strongest effect against yeasts.	*E. coli, S. aureus, Y. lipolytica*	13
WPC-based films incorporated with liquid smoke	liquid smoke	Incorporation liquid smoke into WPC films were effective to prevent *L. monocytogenes* growth, and it may be suitable for food surfaces protection and useful to inhibit *L. monocytogenes*. However, it was not effective to inhibit *E. coli*, *S. aureus*, and *S. Typhimurium*	*Escherichia coli, Staphylococcus aureus, Salmonella typhimurium*, and *Listeria monocytogenes*	74
WPI composite films incorporated with three different types of nano-clays	three nano-clays, Cloisite Na+, Cloisite 20A, and Cloisite 30B.	Incorporation of Cloisite 30B into WPI composite films exhibited higher bacteriostatic effect toward *L. monocytogenes*. The WPI/nano-clay composite films have a great potential for application in food packaging for extending the shelf life, improving quality, and enhancing safety of food packaged	*E. coli O157:H7* and *L. monocytogenes*	78
WPI/Cloisite 30B organo-clay composite films to retard *Listeria monocytogenes*	Cloisite 30B	WPI/Cloisite 30B composite films showed a bacteriostatic effect against *Listeria monocytogenes.*	*L. monocytogenes* and *E. coli* O157:H7.	79
Incorporating oregano oil in WPI films to retard *Pseudomonas*; lactic acid bacteria	oregano oil	Incorporating oregano oil into sorbitol-plasticized WPI films inhibited pseudomonas growth, while lactic acid bacteria growth was completely inhibited. Whey protein films containing oregano oil increase fresh beef shelf life.	*Pseudomonas*; lactic acid bacteria	81

E. coli

WPI films supplemented with *Lb. sakei* cell-free supernatant (containing a bacteriocin-like substance) exhibited antimicrobial activity against *E. coli*, and maintained antimicrobial activity on beef inoculated with *E. coli* [77]. Incorporating organic acids in whey protein film showed antimicrobial activity against *E. coli* [64]. Incorporating of beeswax showed stable antibacterial activity against *E. coli* [43]. Antimicrobial composite edible film containing lysozyme effective to inhibit *E. coli* growth both at the surface and inside region of gouda cheese during ripening[78]. Increasing sodium cyanoborohydride content showed stable antibacterial activity against *E. coli* [79].

L. monocytogenes

Incorporation liquid smoke into WPC films were effective to prevent *L. monocytogenes* growth[76]. Incorporation of Cloisite 30B into WPI composite films exhibited higher bacteriostatic effect toward *L. monocytogenes*[80]. WPI/Cloisite 30B composite films showed a bacteriostatic effect against *Listeria monocytogenes*[81]. WPI films supplemented with *Lb. sakei* cell-free supernatant (containing a bacteriocin-like substance) exhibited antimicrobial activity against *L. monocytogenes* and maintained antimicrobial activity on beef inoculated with *L. monocytogenes*[77].

Listeria innocua

Lactic acid and chitooligosaccharide (COS) in WPI film exhibited bacteriocidal effect on Listeria innocula[13]. WPI-Beeswax, and WPI-Oleic acid film containing lysozyme showed antibacterial activity against *Listeria innocula*[42]. Complexation of lysozyme-polyacrylic acid was an effective tool for controlling *Listeria innocula* growth[14].

Yarrowia lipolytica

Lactic acid and chitooligosaccharide (COS) in WPI film exhibited antimcrobial effect on *Yarrowia lipolytica*. The application on the cheese surface, they more active against bacteria, and less effective toward *Yarrowia lipolytica* and moulds than their commercial coating counterparts[82]. WPI Films formulated with NA was not active toward bacteria, but showed strongest effect against *Yarrowia lipolytica* [13].

Staphylococcus spp.

Antimicrobial whey protein edible film containing lysozyme effective to inhibit *S. aureus* growth both at the inside and surface region of gouda cheese during ripening[78]. WPI film containing lactic acid and chitooligosaccharide showed bacteriocidal effect on *Staphylococcus spp*. and the application on the cheese surface exhibited more active against bacteria but less effective toward yeasts and moulds[82]. WPI Films formulated with lactic acid (LA) and propionic acid (PRO), chitooligosaccharides (COS) and natamycin (NA) showed antimicrobial activity toward *S. aureus* [82]. Incorporation liquid smoke into WPC films was not effective to inhibit *S. aureus* [76].

Pseudomonas spp.

Lactic acid and chitooligosaccharide (COS) in WPI film exhibited bacteriocidal effect on Pseudomonas spp. [82]. Incorporating oregano oil into sorbitol-plasticized WPI films inhibited Pseudomonas growth [82].

Enterobacteriacea

Lactic acid and chitooligosaccharide (COS) in WPI film exhibited bacteriocidal effect on Enterobacteria [82].

Enterococcus

Antimicrobial composite edible film containing lysozyme effective to inhibit Enterococcus [78].

Salmonella Typhimurium

Incorporating organic acids in whey protein film showed antimicrobial activity against *Salmonella* sp. [64].

Yeasts and moulds

Combination sodium benzoate acid and chitooligosaccharide (COS) and combinations of lactic acid and in WPI film exhibited hight inhibitory effect against the yeast. The application on the cheese surface, they more active against bacteria, and less effective toward yeasts and moulds than their commercial coating counterparts [82]. WPI Films formulated NA was not active toward bacteria, but showed strongest effect against yeasts[82]. Antimicrobial composite edible film containing lysozyme effective to inhibit microorganism growth both at the surface and inside region of gouda cheese during ripening [78].

Conclusion

Whey protein edible film containing antimicrobial compound is one of the most interest as biopackaging material due to its ability to retard the growth some bacteria, yeast and mold. Incorporated of antimicrobial compound which attached in some substance such as beeswax, oleic acid and polyacrilic acid can prolong its antimicrobial activity due to controlling the release of antimicrobial compound from whey protein edible film.

References

1. Cha DS, Chinnan MS. Biopolymer-based antimicrobial packaging: a review. Critical Reviews in Food Science and Nutrition., 2004, 44(4): 223-237.
2. Campos CA, Gerschenson LN, Flores SK. Development of edible films and coatings with antimicrobial activity. Food and Bioprocess Technology., 2011; 4, 849–8753.
3. Bourtoom T. Review article: Protein edible film: Properties enhancement. International Food Research Journal, 2009, 16: 1–9.
4. Tharanathan RN. Biodegradable films and composite coatings: Past, present and future. Trends in Food Science and Technology, 2003, 14: 71–78.
5. Tamizharuvi T, Jaisankar V. Antimicrobial film based chitosan/lactic acid showed antimicroal activity against Aspergillus flavus, Candida albicans and Aspergillus niger. International Journal of PharmTech Research. 2015, 8(9): 139-147
6. Ahmad M, Benjakul S, Prodpran T, Agustini TW. Physico-mechanical and antimicrobial properties of gelatin film from the skin of unicorn leatherjacket incorporated with essential oils. Food Hydrocolloids, 2012, 28: 189-199.
7. Debeaufort F, Quezada-Gallo JA, Voilley A. Edible films and coatings: Tomorrow's packaging: Areview. Critical Review in Food Science, 1998, 38, 299-313. In Yousef ARM, Eman AA, Abd El-Moniem, Saleh MMS. The effect of some natural products on storability and fruit properties of Fuerte avocado. International Journal of ChemTech Research, 2015, 8 (4): 1454-1462.
8. Wu Y, Weller CL, Hamouz F, Cuppett SL, Schnepf M, Development and application of multicomponent edible coatings and films: a review. Advances in Food Nutrition Res., 2002, 44, 347-394. In Yousef ARM, Eman AA, Abd El-Moniem, Saleh MMS. The effect of some natural products on storability and fruit properties of Fuerte avocado. International Journal of ChemTech Research, 2015, 8 (4): 1454-1462.
9. Burtoom T, Edible films and coatings: characteristics and properties. Int. Food Res. J., 2008, 15, 237-248. In Yousef ARM, Eman AA, Abd El-Moniem, Saleh MMS. The effect of some natural products on storability and fruit properties of Fuerte avocado. International Journal of ChemTech Research, 2015, 8 (4): 1454-1462.
10. Moneam YKA, Sakr HAA, Ali HE, Khalil SA, El-Naggar AWM, Characterization and Antimicrobial Properties of Gamma Synthesized Plasticized Starch/Poly(vinyl alcohol)/ZnO Composites as Protective Coating for papers. International Journal of ChemTech Research. 2014, 6 (4): 2583-2597

11. Park H.J., Development of advanced edible coatings for fruits. Trends in Food Sci. Technol., 1999, 10,254-260 In Yousef ARM, Eman AA, Abd El-Moniem, Saleh MMS. The effect of some natural products on storability and fruit properties of Fuerte avocado. International Journal of ChemTech Research, 2015, 8 (4): 1454-1462.
12. Gomez-Estaca J, Gimenez B, Montero P, Gomez-Guillen MC. Incorporation of antioxidant borage extract into edible films based on sole skin gelatin or a commercial fish gelatin. Journal of Food Engineering, 2012: 92: 78-85.
13. Ramos, ÓL, Silva SI, Soares JC, Fernandes JC, Poças MF, Pintado ME, Malcata FX, 2012. Features and performance of edible films, obtained from whey protein isolate formulated with antimicrobial compounds. Food Research International, 2012; 45:351–361
14. Ozer B BP, Uz M, Oymaci P, Altinkaya SA. Development of a novel strategy for controlled release of lysozyme from whey protein isolate based active food packaging films. Food Hydrocolloids, 2016: 61: 877-886
15. Mulvihill DM, Ennis MP. Functional milk proteins: production and utilisation. Part B. In Fox PF, McSweeney PLH(Eds.), Advanced dairy chemistry, 2003, 1: 1128-1176. New York, NY, USA: Kluwer.
16. Sothornvit R. Krochta JM, Oxygen permeability and mechanical properties of films from hydrolyzed whey protein. Journal of Agricultural and Food Chemistry, 2000; 48, 3913–3916.
17. Sothornvit R. Krochta JM, Plasticizers in edible films and coatings. In: Han, J.H. (Ed.), Innovations in Food Packaging. Elsevier Academic Press, London, UK, 2005: 403–433.
18. McHugh TH. Krochta JM, Sorbitol-plasticized vs. glycerol-plasticized whey-protein edible films: Integrated oxygen permeability and tensile property evaluation. Journal of Agricultural and Food Chemistry, 1994; 42: 841–845.
19. Khwaldia K, Perez C, Banon S, Desobry S, Hardy J. Milk proteins for edible films and coatings. Critical Reviews in Food Science and Nutrition, 2004; 44: 239–251.
20. Pérez-Gago MB, Krochta JM, Formation and properties of whey protein films and coatings. In A. Gennadios (Ed.), Protein-based films and coatings 2003: 159-177. New York: CRC Press.
21. Krochta JM. de Mulder-Johnston CD. Edible and biodegradable polymer films: Challenges and opportunities. Food Technology, 1997;51: 61–74.
22. Barba C. Andoni E. Juan IM, Preparation and characterization of b-cyclodextrin inclusion complexes as a tool of a controlled antimicrobial release in whey protein edible films. LWT - Food Science and Technology, 2015, 64:1362-1369
23. Gadang VP. Hettiarachchy, NS. Johnson MG. Owens C, Evaluation of antibacterial activity of whey protein isolate coating incorporated with nisin, grape seed extract, malic acid, and EDTA on a Turkey frankfurter system. J. Food Sci., 2008, 73 (8): M389-M394.
24. Pintado CMBS, Ferreira MASS, Sousa I. Control of pathogenic and spoilage microorganisms from cheese surface by whey protein films containing malic acid, nisin and natamycin. Food Control, 2010, 21: 240-246.
25. Fern_andez-Pan I, Royo M, Mat_e JI. Antimicrobial activity of whey protein isolate edible films with essential oils against food spoilers and foodborne pathogens. J. Food Sci. 2012, 77 (7): M383-M390.
26. Appendini P, Hotchkiss JH. Review of antimicrobial food packaging. Innovative Food Science & Emerging Technologies, 2002, 3(2): 113-126.
27. Cagri A, Ustunol Z, Ryser ET. Antimicrobial edible films and coatings. Journal of Food Protection, 2004, 67(4): 833-848.
28. Krasniewska K, Gniewosz M. Substances with antibacterial activity in edible films e a review. Polish Journal of Food and Nutrition Sciences, 2012, 62(4): 199-206.
29. Kuorwel KK, Cran JM, Sonneveld K, Miltz J. Bigger S. Antimicrobial activity of biodegradable polysaccharide and protein-based films containing active agents. Journal of Food Science, 2011, 76: R90-R102.
30. Pérez LM, Soazo MdV, Balagué CE, Rubiolo AC, Verdini RA. Effect of pH on the effectiveness of whey protein/glycerol edible films containing potassium sorbate to control non-O157 shiga toxin-producing Escherichia coli in ready-to-eat foods. Food Control, 2014, 37: 298-304
31. Rocha M, Ferreira FA, Souza MM, Prentice C. Antimicrobial films: a review. In A. M_endez-Vilas (Ed.), Microbial pathogens and strategies for combating them: Science, technology and education, 2013: 23-31. Badajoz, Spain: Formatex Research Center.

32. Suppakul P, Miltz J, Sonneveld K, Bigger SW. Active packaging technologies with an emphasis on antimicrobial packaging and its applications. Journal of Food Science, 2003, 68: 402-420.
33. Cagri A, Ustunol Z, Ryser ET. Antimicrobial edible films and coatings. Journal of Food Protection, 2004; 67, 833–848.
34. Min S, Harris J, Krochta JM. Antimicrobial effects of lactoferrin, lysozyme, and the lactoperoxidase system and edible whey protein films incorporating the lactoperoxidase system against Salmonella enterica and Escherichia coli O157:H7. Journal of Food Science, 2005, 70: 332-338.
35. Tharanathan RN. Biodegradable films and composite coatings: past, present and future. Trends in Food Science and Technology, 2003, 14: 71-78.
36. Pranoto Y, Salokhe VM, Rakshit SK. Physical and antibacterial properties of alginate-based edible film incorporated with garlic oil. Food Research International, 2005, 38: 267–272.
37. Salmieri S, Lacroix M. Physicochemical properties of alginate/polycaprolactone-based films containing essential oils. Journal of Agricultural and Food Chemistry, 2006, 54: 10205–10214.
38. Chen W, Walker S, John CB. The mechanism of floc formation in protein precipitation by polyelectrolytes. Chemical Engineering Science, 1992: 47(5): 1039-1045.
39. Ghimire A, Kasi RM, Kumar CV. Proton-coupled protein Binding: Controlling lysozyme/poly(acrylic acid) interactions with pH. The Journal of Physical Chemistry B, 2014, 118(19): 5026-5033.
40. Kamiya N, Klibanov AM. Controlling the rate of protein release from polyelectrolyte complexes. Biotechnology and Bioengineering, 2003, 82(5): 590-594.
41. Kim, W.-S., Hirasawa, I., and Kim, W.-S. (2002). Aging characteristics of protein precipitates produced by polyelectrolyte precipitation in turbulently agitated reactor. Chemical Engineering Science, 2002; 57(19): 4077-4085.
42. Boyacı DF. Yemenicioglu KA, Development of activate-at-home-type edible antimicrobial films: An example pH-triggering mechanism formed for smoked salmon slices using lysozyme in whey protein films. Food Hydrocolloids 2016; 60: 170-178
43. Apriliyani WA, Rosyidi D, Purwadi, Purnomo H, Manab A. The Release of Egg White Lysozyme Containing EDTA from Composite Edible Film Based on Whey Protein, Konjac Flour and Lipid. Advance Journal of Food Science and Technology, 2014, 6(1): 48-55
44. Mecitoglu, Ç, Yemenicio_glu A, Arslano_glu A, Elmacı ZS, Korel F, Çetin AE. Incorporation of partially purified hen egg white lysozyme into zein films for antimicrobial food packaging. Food Research International, 2006, 39(1): 12-21.
45. Unalan IU, Korel F, Yemenicio_glu A. Active packaging of ground beef patties by edible zein films incorporated with partially purified lysozyme and Na2EDTA. International Journal of Food Science & Technology, 2011, 46(6): 1289-1295.
46. Marolia KZ, D'Souza SF, Enhancement in the lysozyme activity of the hen egg white foam matrix by cross-linking in the presence of N-acetyl glucosamine. *J Biochem Biophys Methods*, *39* (1-2): 115-7. In Vijitha M, Gomathi S, Rathnasamy SK, Separation and Purification of lysozyme from Emu (Dromaius novaehollandiae) Egg White and its antimicrobial Characterization. International Journal of PharmTech Research, 1999, 6 (4): 1292-1300.
47. Hawkins CL, Davies MJ, Inactivation of protease inhibitors and lysozyme by hypochlorous acid: role of side-chain oxidation and protein unfolding in loss of biological function. *Chemical research in toxicology*, *18* (10): 1600-10. In Vijitha M, Gomathi S, Rathnasamy SK, Separation and Purification of lysozyme from Emu (Dromaius novaehollandiae) Egg White and its antimicrobial Characterization. International Journal of PharmTech Research, 1999, 6 (4): 1292-1300.
48. Appendini P, Hotchkiss JH. Immobilization of lysozyme on food contact polymers as potential antimicrobial films. Packaging Technology and Science, 1997, 10(5): 271-279.
49. Shah NP, Effects of milk-derived bioactives: an overview. British Journal of Nutrition, 2000, 84(S1), 3-10.
50. Gomathi S, Vijitha M, Rathnasamy SK, Affinity Separation of Lysozyme from Quail Egg (Coturnix ypsilophora) and its Antimicrobial Characterization. International Journal of PharmTech Research, 2014, 6 (4): 1286-1291
51. Alvarez-Ord_o~nez A, Leong D, Hickey B, Beaufort A, Jordan K. The challenge of challenge testing to monitor Listeria monocytogenes growth on ready-to-eat foods in Europe by following the European Commission. Technical Guidance document. Food Research International, 2015, 75: 233-243.

52. Beristain-Bauza SC, Mani-L_opez E, Palou E, L_opez-Malo A, 2016. Antimicrobial activity and physical properties of protein films added with cell-free supernatant of Lactobacillus rhamnosus. Food Control, 2016, 62: 44-51.
53. Aksu MI, Kaya M, Oz F, Effect of Lactobacillus sakei and Staphylococcus xylosus on the inhibition of Escherichia coli O157:H7 in pastirma, a dry-cured meat product. J. Food Saf, 2008, 28, 47-58.
54. Beristain-Bauza SdC, Mani-Lopez E, Palou E, Lopez-Malo A. Antimicrobial activity of whey protein films supplemented with Lactobacillus sakei cell-free supernatant on fresh beef. Food Microbiology, 2017, 62: 207-211
55. Luck E, Jager M. Antimicrobial food additives: characteristics, uses, and effects. 1997. 2nd ed. Berlin: Springer Verlag.
56. Pérez LM, Balagué CE, Rubiolo AC, Verdini RA. Evaluation of the biocide properties of whey-protein edible films with potassium sorbate to control non-O157 shiga toxin producing *Escherichia coli.* Procedia Food Science, 2011, 1: 203 – 209
57. Perez LM, Soazo M, Balague CE, Rubiolo AC, Verdini RA. Effect of pH on the effectiveness of whey protein/glycerol edible films containing potassium sorbate to control non-O157 Shiga toxin-producing Escherichia coli in ready-to-eat foods. Food Control, 2014, 37, 298-304.
58. Cagri A, Ustunol Z, Ryser, ET. Antimicrobial, mechanical, and moisture barrier properties of low pH whey protein-based edible films containing paminobenzoic or sorbic acids. Journal of Food Science, 2001, 66(6): 865-871.
59. Hauser C, Wunderlich, J. Antimicrobial packaging films with a sorbic acid based coating. Procedia Food Science, 2011, 1, 197-202.
60. Davidson MP, Juneja V, Banen J, 2001 In J. H. Thorngate, Iii, S. Salminen, L. A. Antimicrobials in food additives, 2001. Branen, & M. P. Davidson (Eds.),
61. Guillard V, Issoupov V, Redl A, Gontard N. Food preservative content reduction by controlling sorbic acid release from a superficial coating. Innovative Food Science and Emerging Technologies, 2009, 10: 108-115.
62. Silveira MFA, Soares NFF, Geraldine RM, Andrade NJ, Botrel DA, Gonçalves MPJ. Active film incorporated with sorbic acid on pastry dough conservation. Food Control, 2007; 18: 1063-1067.
63. Bottcher H, Jagota C, Trepte J, Kallies KH, Haufe H. Solegel composite films with controlled release of biocides. Journal of Controlled Release, 1999: 60, 57-65.
64. Manab A, Sawitri ME, Al-Awwaly KU, Purnomo H. Antimicrobial activity of whey protein based edible film incorporated with organic acids. African Journal of Food Science, 2011, 5 (1): 6-11.
65. Alakomi HL, Skytta E, Saarela M, Mattila-Sandholm T, Latva-Kala K, Helander IM. Lactic acid permeabilizes Gram-negative bacteria by disrupting the outer membrane. Applied and Environmental Microbiology, 2000, 66: 2001–2005.
66. Dibner JJ, Butin P. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. Journal of Applied Poultry Research, 2002, 11: 453–463.
67. Ray B. Fundamental Food Microbiology (3rd ed.). 2004; Boca Ratton FL, USA: CRC Press.
68. Lingbeck JM, Cordero P, O'Bryan CA, Johnson MG, Ricke SC, Crandall PG. Functionality of liquid smoke as an all-natural antimicrobial in food preservation. Meat Science, 2014, 97(2), 197-206.
69. Vitt SM, Himelbloom BH, Crapo CA. Inhibition of Listeria innocua and Listeria monocytogenes in a laboratory medium and cold-smoked salmon containing liquid smoke. Journal of Food Safety, 2001, 2: 111-125.
70. Holley RA, Patel D, Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. Food Microbiology, 2005, 22: 273-292.
71. Milly PJ, Toledo RT, Ramakrishnan S. Determination of minimum inhibitory concentrations of liquid smoke fractions. Journal of Food Science, 2005, 70(1): M12-M17.
72. Gedela S, Escoubas JR, Muriana PM. Effect of inhibitory liquid smoke fractions on Listeria monocytogenes during long-term storage of frankfurters. Journal of Food Protection, 2007, 70: 386-391.
73. Martin EM, O'Bryan CA, Lary RYJr, Griffis CL, Vaughn KL, Marcy JA. Spray application of liquid smoke to reduce or eliminate Listeria monocytogenes surface inoculated on frankfurters. Meat Science, 2010, 85: 640-644.
74. Morey A, Bratcher CL, Singh M, McKee SR. Effect of liquid smoke as an ingredient in frankfurters on Listeria monocytogenes and quality attributes. Poultry Science, 2012; 91(9): 2341-2350.

75. Moore TVL, Moore DKJr. Protective film peelably adhered to edible film enclosing product. 1998; United State Patent US 5741533 A.
76. Soazo M, Perez LM, Piccirilli GN, Delorenzi NJ, Verdini RA. 2016. Antimicrobial and physicochemical characterization of whey protein concentrate edible films incorporated with liquid smoke. LWT - Food Science and Technology, 2016, 72: 285-291
77. Beristain-Bauza, S. d. C., E. Mani-Lopez, E. Palou, A. Lopez-Malo, 2017. Antimicrobial activity of whey protein films supplemented with Lactobacillus sakei cell-free supernatant on fresh beef. Food Microbiology 62: 207-211
78. Haniyah YS, Purwadi, Radiati LE, Thohari I, Setyowati E, Manab A. 2016. Gouda Cheese Microbial Controlling During Ripening Using Composite Edible Film Containing Lysozyme. *Int.J.Curr.Microbiol.App.Sci*, 2016, *5*(5): 748-756
79. Manab A, Sawitri ME, Al-Awwaly KU, Widati AS, Haniyah YS. 2016. Antibacterial and Physical Properties of Composite Edible Film Containing Modified Lysozyme and Sodium Cyanoborohydrate. International Journal of ChemTech Research, 2016, 9(4): 421-429.
80. Sothornvit R, Rhim JW, Hong SI. 2009. Effect of nano-clay type on the physical and antimicrobial properties of whey protein isolate/clay composite films. Journal of Food Engineering; 2009, 91 468–473
81. Sothornvit R, Hong SI, An DJ, Rhim JW. 2010. Effect of clay content on the physical and antimicrobial properties of whey protein isolate/organo-clay composite films. LWT - Food Science and Technology, 2010, 43: 279–284
82. Ramos ÓL, Santos AC, Leão MV, Pereira JO, Silva SI, Fernandes JC, Franco MI, Pintado ME, Malcata FX. Antimicrobial activity of edible coatings prepared from whey protein isolate and formulated with various antimicrobial agents. International Dairy Journal 2012a; 25: 132-141.
83. Zinoviadou KG, Konstantinos P, Koutsoumanis, Biliaderis CG. Physico-chemical properties of whey protein isolate films containing oregano oil and their antimicrobial action against spoilage flora of fresh beef. Meat Science, 2009, 82: 338–345

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