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PRODUCTION OF EDIBLE COATING FROM WHEY PROTEIN

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ABSTRACT

whey protein isolate –based edible film (at pH 5.2) containing sorbic acid (S A) or p- amino benzoic acid (PABA) were developed and assessed for inhibition of the growth of Aerobic and / or Enterobacteriacea bacteria . Water vapor permeability (WVP), tensile strength (TS) , and percent elongation (%E) were determined . Using 1.5 % SA and PABA a average inhibition reached 71% , 53% respectively .Addition of SA and PABA increased %E , but decreased TS. WVP was not affected by 0.5 % and 0.75% SA; however, PABA increased WVP.

INTRODUCTION

One of the most useful functions of edible films is their ability to act as barriers, either to gas, oil , or , more often water . Moisture levels in foods are critical for maintaining freshness, controlling microbial growth, and providing mouth feel and texture. Edible films can control water activity preventing either moisture loss or uptake. Second, edible coatings are applied to the surface of foods and crackers to serve as a foundation or adhesive for goings. Edible films can also act as glazes to enhance appearance of baked goods. Hat yai,. (2008).

During the last thirty years, considerable research work aimed at the development of edible packaging films and coatings has been conducted. However, few of these films have been applied commercially. This fact can be attributed mainly to the limitations of the films in comparison with traditional polymer films. The polymer industry has been able to provide food industry process with a wide variety of packaging materials characterized generally by better physical and barrier properties than the edible films. Research on edible films continues and seems to have intensified over the last few years. Considering a number of advantages these films have over polymeric materials, one might. Anticipate that the future in food packaging belongs to edible films .Krochta *et al*. (1990)

Application of various types of edible coating as well as multi component edible coatings systems, on meat and sea food is reviewed. Aspects considered include : rationale of using edible coatings on meat and sea foods ; lipid – based coatings (waxes , fats and oils ; glycerides and acetylated glycerides) : poly saccharide – based coatings (starch and starch derivative , alginates , carrageenans , agar , dextran , cellulose ethers) and protein – based coatings (collagen , gelatin , milk proteins , cereal proteins , oilseed proteins , improvement of protein film properties .Thawien,.(2012).

Increased consumer demands for both higher quality and longer shelf life foods in combination with environmental needs for reduction of disposable packaging amounts and improvement in packaging recyclability have led to increased interest in edible film research. Krochta,. (1992).

Edible Films, by regulating water vapor, oxygen, carbon dioxide, and lipid transfer, in food systems, offer potential solutions to these concerns. Edible films can also improve food system mechanical properties and control the loss of volatile flavors and aromas. Researchers are now investigating proteins, lipids, and polysaccharides, both alone and in combination, as to their effectiveness as edible masses transfer barriers of these categories, proteins have been the least studied . Atomizing spray systems for application of Edible coatings by Ricardo, *et al*., (2012)

The unique characteristics of milk whey proteins make them excellent candidates for incorporation into edible films and coatings to control mass transfer in food systems. Whey proteins represent 20 % of the total milk proteins. Jovanovic,.*et al*,.(2005).

They are characterized by their solubility at(pH 4.6). Whey protein contains five protein types : Lactalbumin , B- lactoglobulin , x-lactalbumine , bovine serum albumin , immunoglobulin's , and protease peptones .

Liquid whey is a byproduct of cheese manufacture and the annual production of fluid whey is rising. B- Lacto globulin in the major protein in the whey fraction (62%) McHugh, *et al*., (1994). Efficient purification procedures for whey protein isolates (WPI) and why protein concentrates (WPC) have been developed. Ultra filtration techniques are currently employed to isolate undenatured WPCs. High performance hydrophilic ion exchange is used to purify WPIS. WPCs range from 25% to 80% whey protein, whereas, WPIs have protein contents greater than 80% .Qi ,P,X., *et al*,. (2011).

An objective of our study was to define and optimize conditions necessary for the formation of whey protein edible films. Another objective was to examine the effects of PH, plasticizers, and relative humidity on the water vapor permeability of such films .

MATERIALS AND METHODS

Calcium chloride ,Sodium hydroxide , Magnesium chloride ,Potassium Carbonate ,Sodium bromide ,Sodium chloride ,Calcium ascorbate and Lithium chloride were purchased from Brillocera ,S.A (Valencia ,Spain).Sorbic acid ,Hydrochloric acid,Ascorbic acid and teflon plates were from Panreac quimic,S.A(Barcelona ,Spain).

Film materials:

Whey protein isolate, sorbitol, glycerol and polyethylene glycol plasticizers were used. Lithium chloride, magnesium chloride, potassium carbonate , sodium bromide and sodium chloride salts were used for formation of saturated salt solutions . Sodium hydroxide , hydrochloric acid , ascorbic acid and calcium ascorbate were used for PH adjustment of film solutions . (All chemicals were laboratory grade) . whey protein isolate was supplied by le Sueur isolates (le Sueur , NN) .

Film Formation:

Whey protein isolate (WPI , Alacen 895) (8%w/v) were dissolved in distilled water containing 0.04% CaC12 (w/v) . After mixing and adjusting the PH to 8.0 with 1.0 N NaoH , the solution was heated at 90 c for 30 min in a

shaking water bath . Following the addition of candelilia wax (stahl pash Inc., New York , N.Y., U.S.A) (0.4%w/v) during the last 5 min of heating , the solution was homogenized for 2 min at 13.500 rpm in a SD-45 homogenizer , filtered through cheese cloth and cooled to 23+2C . After incorporating 0.5 %, 0.75 % , 1.0 % or 1.5 % (w/v) sorbic acid (SA) or p-amino – benzoic acid (PABA) , the PH was adjucted to 5.2 (control films of this study formate without antimicrobials) .Yoo S,.*et al*.(2011).

Weights of glycerol (GLY) , polyethylene glycol (PEG) or sorbitol (S) relative to the weight of WPI originally dissolved were then added as plasticizers for films (e.g., equal weights of WPI and S yields(50% WPI / 50% S films) . The whey protein / glycerol or sorbitol or polyethylene glycol solutions were than reheated to 75 C and rehomogenized for 2 min. Also at 13.500 rpm, followed by 4 min. at 20.500 rpm., filtered through cheese cloth and cooled to 23 + 2 C) . Following degassing by vacuum to remove dissolved air , the whey protein solution (40ml/ plate) was cast by pipe ting the solution into sterile 17-cmdia Teflon plates . The solutions were dried for approximately 24 h at 23 + 2C and 50 % + 5% relative humidity (RH) , after which the films were peeled from the plates and stored at 23 + 2 c and 50% + 5 % RH until used , according to Mchugh,et a.,(1994).

1-Total count of bacteria:

Aerobic colony count and Enterobacteriaceae count were carried out according to Chen, *et al*., (1996) .

2-Film thickness :

Thicknesses of films were measured with a micrometer (L.S. Starett co., Series 436 , catalog No. T436 RL. 1, Athol MA.) to the nearest 0.002 mm at five random positions around the film individual film thickness measurements varied up to 5% . Average values of five thickness measurements . Film were used in all water vapor permeability(wvp) calculations, according to De Wit,(1981).

3-water vapor permeability determination :

Water vapor permeability (wvp)was determined using the WVP correction method described by Mchugh, *et al*., (1993) . To measure water vapor permeability with poly-(methy1 methacrylate) test cells . Distilled water (7 ml) was placed in each test cell to expose the film to high percentage of relative humidity (RH)on one side through a circular opening of 5 cm diameter . Four 6.5-cm – diameter films of each formulation were sealed in the test cells using a silicone sealant to avoid vapor leaks through cell joints. The side of the film facing the high percentage of RH was noted. WVP was calculated from water vapor transmission rate through film, the partial vapor pressure difference between the two sides of the film and thickness according to McHugh., *et al*(1993) .

4- Mechanical properties (MP):

Films were cut into strips measuring 101.6mm by 25.4mm using a precision sample cutter (Thawing Albert Instrument Co., Philadelphia , Pa ., U.S.A) . All films were conservation for 48 h at 23+ $2C^{o}$ / 50% + 5% RH before testing Tensile strength (TS) and percent elongation at break were determined according to ASTM (1992) . The test was run using the Instron universal Test Machine Model 2401 (canton , Mass., USA) at 23±$2C^{O}$ / 50 %

± 5 % RH with a static load cell of 1 KN and a cross high speed of 50.8 cm / min . TS was calcuted in MP from following equation:
TS = load / sample X sample thickness
% Elongation at break was determined by the follow equation
% E= (distance sample stretched / original length of sample) × 100

Results and Discussion

1-Mechanical properties :

From Table (1) we can show that the average film thickness was 127.01 Um with no significant differences observed between films (Table 1) . When SA and PABA concentrations increased from 0% to 1.5 % , % E increased from 6.39% to 74.27% and 41.17%, respectively (Table 1) . While TS of WPI films significantly decreased with increasing levels of SA or PABA

(Table 1) Mechanical properties of whey proteins isolate-based films:

PRoPERTIES / additves	Antimicrobial % (w/v)	Thickness (UM)	(Elongation) %(E)	TS(MPa) (Tensile strength)	WVP (Water vapor permeability) (g.mm/m2.d.k.po)
SA	0.00%(control)	127.01	6.39	5.90	27.20
	0.50%	121.35	26.35	4.49	27.28
	0.75%	137.24	36.56	3.80	27.58
	1.00%	130.23	73.53	3.71	43.57
	1.50%	120.57	74.27	2.69	45.59
PABA Additives	0.50%	123.04	18.38	5.30	51.78
	0.75%	134.12	20.86	5.22	54.90
	1.00%	131.19	33.28	5.38	55.34
	1.50%	120.32	41.17	5.79	55.39

***MPa: mill paskal unit**
*** UM: micro meter**

Tensile strength(TS) of films containing 1.5% PABA (5.79MPa) was nearly to the control (5.90MPa) . Films containing SA exhibited lower TS and higher % E as compared to films containing PABA. The reason for this phenomenon could be that the straight chain of SA can more easily penetrate into WPI chains than PABA , which has a benzene ring . consequently , SA may have allowed more mobility between protein chains , thereby producing films of lower TS and greater flexibility .

Increasing the amount of additives other than cross linking agents generally produced films with lower TS and greater elongation , since these molecules insert between protein chains to form hydrogen bonds with amide groups of proteins (Kester and Fennema 1986) . Reduced interaction between these protein chains lead to increase flexibility and movement . In our study , SA and PABA might be functioning as plasticizers to increase elongation and decrease TS . Calcium chloride (Cacl2)was incorporated into our film solution as cross linking agent to improve the mechanical and water vapor permeability properties of the low PH films as previous suggested by others (Avena –Bustillos and Krochta,(1993). As a divalent action , calcium crosslinks between negatively charged groups on proteins , thereby increasing cohesion between protein chains , reducing protein polymers segmental mobility and improving both the mechanical properties and water

vapor permeability (Jeyarajah and Allen 1994) . They reported that Cacl2 induce a change in B-lactoglobulin conformation, which facilitate polymerization during heating . calcium ions also increasing the reactivity of SH groups at low PH , aggregation of most whey proteins can still occur in the presence of calcium. De Wit,(1981)

Whey protein films are formed by heat –catalyzed protein – protein interactions that involve disulfide , hydrogen , hydrophobic bonds . Heating denatures the protein and expo-internal SH and hydrophobic groups (shimada and Cheftel 1998) , which promoto intermolecular S-S and hydrophobic bonding upon drying (McHugh and krochta ,(1994). film formation is favored more alkaline film solutions since SH reactivity increases PH > 8 (Banerjee and Chen 1995) . The present study , the film solution was at PH 8.0 during heating at 90c , after which the PH was decreased to 5.2 using lactic and acetic acid . Alow PH environment would likely prevent S-S bond formation in the protein matrix , thereby weakening the film structure . Thus , tensile strength of the low – PH film (5.90 MPa) was substantially lower than that reported for high- PH film (13.9 MPa) (McHugh and Krochta 1994 a). However , tensile strength of our low – PH film was higher than the reported for corn zein (o.4 MPa) . soya protein (4.5 MPa) (Gennadios,*et al*., 1991) and wheat gloten based edible films (1.9 to 4.4 MPa) (Gennadios ,*et al*., 1993) when tested at 23C^{O}/ 50 % RH .

2- Water vapor permeability: (WVP)

Film containing 0%, 0.5 %, 0.75 %, 1.0 % or 1.5 % of PABA inhibited the average values of WVP values of 27.20, 51.78, 54.90, 55.34, and 55.39 at(g.mm/ m2 .d.kpa) , respectively (Table 1) . Increasing the concentration of PABA from 0.5 % to 1.5 % did not significantly after WVP. WVP values for films containing 0.5% and 0.75% SA were 27.28 and 27.58 g .mm /m2.d.kpa, respectively, and were not significantly different at the control (27.20g.mm/m2.d.kpa) (PABA) . However addition of 1.0 % and 1.5 % SA significantly increased WVPto 43.57 and 45.59 g.mm/m2.d.kpa, respectively. WVP is a measure of the ease with which a material can penetrate by water vapor. WPI edible films tend to be more moisture barrier due to a abundant hydrophilic groups proteins. Their moisture barrier properties can be improved by adding no polar compounds such as lipids (McHugh and Krochta ,1994 b) . We incorporated candeilla into the film solution to reduce WVP . In preliminary experiments, diffusion of SA and PABA as demonstrated by inhibition zones was similar for films prepared with and without candelilla wax (results not shown). Adding SA and PABA to the film solution increased WVP because both antimicrobials are hydrophilic compounds. Addition of polar additives may increase the hydrophilic character and the mobility coefficient of the film .McHugh , *et al*., (1994) . Moreover, additives such as SA or PABA weaken chain linking in the film to produce a looser structure, which increase water mobility.

Antimicrobial properties :

Increasing the concentration of PABA and SAincreasing the inhibition the growth of aerobic colony and Enterobacteriaceae counts (Table 2) due to their increased ability to penetrate the cytoplasmic membrance of bacteria at PH 5.2 (Tsai , and Chou, (1996) . Control films without antimicrobials were

non – inhibitory. Therefore, films containing antimicrobial in our study would be best suited for foods that have values near PH 5.2 , such meats and cheeses .

Films containing SA were generally more inhibitory to Aerobic bacteria than films contain PABA but the films containing PABA were more effective against Enterobacteriaceae SA. Richards,*et al*.,(1994) and Tsai andChou,(1996) showed similar inhibition of E.coli on laboratory media using PABA and SA, respectively .

Table(2): Antimicrobial activites of whey protein isolate – based edible films containing sorbic acid or p-amino benzoic acid .

Treatments / Kind Of Microbe	Film without antimicrobial microbe/gm	Films with antimicrobials							
		PABA				SA			
		0.5%	0.75%	1.0%	1.5%	0.5%	0.7%	1.0%	1.5%
Aerobic colony	90.000/ gm	70.200	60.300	46.800	43.200	62.100	45.900	31.500	26.100
Percent of decreasing		*22%	*33%	*48%	*52%	*31%	*49%	*65%	*71%
Enterobacteriaceae count	10.000/ gm	7.200	5.600	3.800	3.000	8.100	7.100	5.600	4.700
Percent of decreasing		*28%	*44%	*62%	*70%	*19%	*29%	*44%	*53%

***Percent of decreasing (Inhibition)**

Conclusion

Adding 0.5% to 1.5% of SA or PABA into WPI films (PH 5.2) led to inhibition of Aerobic and Enterobacteriaceae microbes at PH 5.2. Addition of PABA and SA increased % E and WVP , but decreased TS(Table1). Given any current works involving ready to – eat meat products, which will be reported elsewhere, these films may prove useful for inactivating post-processing contamination on ready – to eat foods such as processed meats.

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انتاج أغشية غذائية من بروتينات شرش اللبن

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تم استخدام بروتينات شرش اللبن في انتاج أغشية غذائية عند (PH5.2) مدعما بحامض السوربيك وحامض امينوبنزويك وذلك لتحسين تثبيط النمو الميكروبي للبكتريا الهوائية وبكتريا الانتيروباكتيريسي .

وتم دراسة درجة نفاذية بخار الماء ، قوة الشد ، والنسبة المئوية للاستطاله باستخدام (1.5%) من كلا من حامض السوربيك وحامض الامينو بنزويك وقد وصلت نسبة التثبيط الميكروبي إلي (71%) في حالة حامض السوربيك ووصلت إلي نسبة (53%) في حالة الامينو بنزويك

وباضافة حامض السوربيك وحامض الامينو بنزويك رفع من النسبة المئوية للاستطاله ، بينما خفض من قوة الشد للغلاف .

بينما لم تتأثر نفاذية الماء عند تركيز (0.5%) ، (0.75%) من حمض السوربيك . بينما رفع حامض الامينو بنزويك من نفاذية بخار الماء .

قام بتحكيم البحث

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