CEREAL BIOPOLYMER FILMS, COATINGS AND OTHER INDUSTRIAL PRODUCTS

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Many polysaccharides and proteins form materials with useful material properties. Materials from native biopolymers generally have excellent gas barrier properties, good mechanical properties, and form appealing transparent films, which are often even thermoplastic.

Thermoplastic proteins can be prepared by using a suitable plasticizer and by using a proper combination of mechanical shear and heat. The amount of plasticizer and the processing conditions leads to materials with different chemical and physical properties which can be optimized to produce films, fibers and foams. The effect of type and amount of plasticizer, and the effect of processing conditions on the thermal and rheological properties of thermoplastic proteins will be presented.

Edible coatings and films can be applied on fresh and minimally processed fruits and vegetables to extend their shelf life by creating a modified atmosphere and preventing water loss. White discoloration, a physiological defect in minimally processed carrots was reduced by the use of a cellulose based coating. However, higher incidence of microbiological spoilage in coated carrots suggests the importance of antimicrobial inclusion in coatings.

Edible coatings and films can be utilized as carrier for preservatives, anti-oxidants, flavours etc. Films of potato starch have been used as carriers for preservatives and the anti-microbial effect on a contaminated model food was significant. Several species of bacteria, yeast and moulds were investigated together with different preservatives. The anti-microbial protection was most efficient on bacteria and least efficient for moulds.

INTRODUCTION

Cereal biopolymers such as proteins and polysaccharides are attractive raw materials for use as materials in packaging applications. The raw materials are inexpensive, produced from renewable resources, edible and biodegradable. The materials have in general good mechanical properties and are excellent gas and grease barriers[1-4]. They can often be processed thermoplastically and these materials can also be used in packaging as an edible coating directly applied on food.

Edible coatings and films can be applied on fresh and minimally processed fruits and vegetables to extend their shelf life by creating a modified atmosphere and preventing water loss. Fresh fruits and vegetables deteriorate because of physiological ageing, biochemical changes and microbiological spoilage.[5] Such deterioration is compounded in minimally processed fruits and vegetables (MPFV) because of wounding of the tissues that occurs during unit operations such as peeling, cutting and slicing.[6] The shelf life of MPFV at refrigeration conditions as determined by sensory and microbiological quality can be 7-14 days or less depending on the produce, the storage conditions and associated hurdles.[7] Methods such as refrigeration, use of chemical additives and irradiation have shown potential to extend the shelf life of MPFV, but new approaches include creating a modified atmosphere using edible coatings/biofilms and polymeric packaging films.[6] Minimally processed carrots (MPC) prepared by peeling and slicing is one of the major MPFV products that are used as a ready to eat snacks or salad vegetables. The main problems with minimally processed carrots that limit their shelf life are white blush discolouration, a visual defect and microbiological spoilage. The white blush discolouration has been attributed to a reversible change because of surface dehydration[8] as well as an irreversible change attributed to lignification by enzymatic processes[9]. Minimally processed carrots were found to have a typical lactic acid fermentation type of spoilage by the lactic acid bacteria.[10]

An increasing interest is today devoted to the possibility of using manufacturing technologies, employed for conventional thermoplastic polymers, for polysaccharides and proteins. In comparison to these natural occurring polymers, thermoplastic synthetic polymers can be melt processed by simply applying heat and shear. Unfortunately, the high crystallinity and the strong intermolecular interactions present in proteins and polysaccharides leads to thermal degradation of the material, without achieving melt flow. The latter is however possible if there is a suitable plasticizer present, used in combination with heat and shear, that can act as internal lubricant leading to enough molecular mobility of the macromolecules, necessary for melt flow.

In this paper three different examples will be given regarding the use of biopolymers in packaging related applications. Edible barriers on minimally processed carrots and other fruits and vegetables, release from biopolymer coatings and the physical-chemical and mechanical properties of a thermoplastic biopolymer are highlighted.

BIOPOLYMER BARRIERS ON MINIMALLY PROCESSED CARROTS, AND OTHER FRUITS AND VEGETABLES

The effects of applying Nature Seal®, cellulose based coating on the sensory and microbiological quality of packaged MPC was studied over a period of 12 days at 10 °C. The white blush formation was quantified by whiteness Index[9] and lactic acid bacteria were enumerated using lactobacillus MRS agar[11]. The results showed that white blush formation increased with storage time, but it was significantly ($P<0.05$) controlled by the coating treatments (Table I). Polysaccharide coatings can act as sacrificing agents to water loss[12] and can hold water on the surface for a longer period[13]. Thus, the coating retarded the surface dehydration, which is the main cause of white blush formation. When whiteness index was used as shelf life indicator[8], at 10 °C a shelf life extension from 3 to 5-6 days was achieved for the coated MPC compared with the uncoated ones.

Time (days)	Coating (C)[b]		
	c0 **(control)**	c75 (75 mgg^{-1} coating)	c150 **(150 mgg^{-1} coating)**
0[c]	37 (±0.59)	37 (±0.59)	37 (±0.59)
4	47 (±1.28)	44 (±2.01)	46 (±1.49)
8	48 (±2.42)	47 (±0.60)	46 (±1.40)
12	51 (±1.36)	49 (±0.91)	47 (±0.76)

[a] Each value represents a mean of four replicates with standard deviations in bracket, and the overall effect of the variables was derived from the table of means of the statistical analysis

[b] Values in this column indicate concentrations of NatureSeal CA1 in coatings

[c] d0 is taken as a reference value of fresh carrots prior to treatment

Table I Effect of coatings on the whiteness index scores[a] of sliced carrots packs stored at 10 ^{0}C for 12 days

However, with storage time, the coatings enhanced the growth of lactic acid bacteria (Table II). The coating in the research carried out here could have promoted some favourable conditions for the microbes to proliferate by providing some nutrients for their growth. In addition the coating might have provided a higher water activity on the surface of the carrots to enhance microbiological growth and spoilage. Baldwin, Nisperos-Carriedo, Chen and Hagenmaier[14] found that it is important to incorporate sodium benzoate and potassium sorbate in coatings to effectively control the microbial spoilage of cut apple and potato.

The application of other edible coating, namely protein-based ones have shown potential in enhancing quality of fruits and vegetables. Zein, maize prolamin protein was reported to delay ripening of tomatoes by 6 days[15]. In another study, plasticized zein films with oleic acid have shown to maintain the original firmness and colour of fresh broccoli over a storage period of 6 days compared with the control[16]. This extension of the shelf life was attributed to creation of modified atmosphere by the zein films. As Zein protein is extensively homologous to the kafirin prolamin from sorghum[17], it can be proposed that kafirin may have good potential as biofilms/coatings.

Time (days)	Coatings[b]		
	c0 **(control)**	c75 **(75 mgg^{-1} coating)**	c150 **(150 mgg^{-1} coating)**
0[c]	2.39 (±0.02)	2.39 (±0.02)	2.39 (±0.02)
4	3.32 (±0.26)	4.17 (±0.37)	3.62 (±0.35)
8	3.63 (±0.39)	5.24 (±0.26)	5.92 (±0.47)
12	4.32 (±0.56)	5.07 (±0.60)	5.18 (±0.47)

[a] Each value represents a mean of four replicates with standard deviations in bracket,

[b] Values in this column indicate concentrations of NatureSeal CA1 in coatings

[c] d0 is taken as a reference value of fresh carrots prior to treatment

Table II The effects of coatings on growth of lactic acid bacteria (log cfu g^{-1})[a] of minimally processed carrot packs stored at 10 ^{0}C for 12 days

Other applications of edible coatings on fresh and minimally processed fruits and vegetables can be summarized as follows:

- Reduction of weight loss by Nature Seal® coated on cut potato and apple[14] and better browning and microbial control by Nature Seal® adjusted to a pH of 2.5-3.0 with an acidulant, together with preservatives and antioxidant on coated fresh cut potato and apple[14]
- Firmer fruit with high titratable acidity, better colour retention and decrease in respiration rate and fruit decay by the chitosan coating on fresh strawberries[18]
- Significant decrease in inoculated *Salmonella montevideo* by 2 log cycles on fresh tomato skin by cellulose based coating with acetic and citric acid[19]
- Lower respiration rate and better colour firmness retention in green bell peppers with mineral oil based coating[20]

RELEASE FROM BIOPOLYMER COATINGS

The release from potato starch, whey protein and monoglyceride films has been studied using ascorbic acid and amaranth dye as probes[21]. Ascorbic acid is used as a preservative and has a MW of 192. The amaranth dye, MW 604, was selected to mimic higher molecular weight compounds used as additives. A model food consisting of a gelatine/sugar gel with a preset water activity in the range 0.5-0.95 was coated with starch, whey protein or monoglyceride containing the probe or covered with a film of the same materials. The release from the coating to the model food was measured by serial sectioning of 1 µm thick slices, down into a piece of the coated model food. The concentration profile was obtained by measuring the probe concentration in the slices with a spectrophotometer.

The probe diffusion into the model food increased, as anticipated, with increased water activity and decreased with increased molecular weight of the probe. However, the release pattern differed between starch and whey protein coatings, as shown in Fig. 1. The release from the whey protein coatings showed an initial increase in concentration with depth or time, which was attributed to initial swelling of the starch coating. The difference in release pattern between different materials is an important design parameter to control the release.

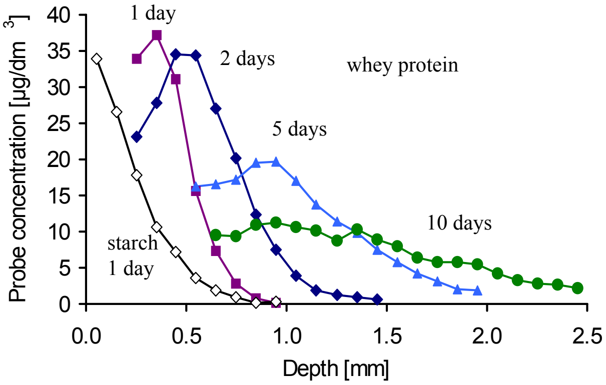

Figure 1 Concentration profile from the release of amaranth dye from coatings of whey protein and starch into a model food, a_w=0.95.

ANTIMICROBIAL EFFECT

Films and coatings containing preservatives have been designed to inhibit proliferation of a surface contaminant[22]. Native potato starch (NPS), whey protein isolate (WPI) and monoglyceride films containing parahydroxybenzoethylester (PHB) were tested in a worst-case scenario. A model food consisting of an agarose gel at a_w=0.95 containing microbial nutrients was infected on the surface with different microorganisms, se Table III. Spores of the microorganisms were used where applicable. The infected surface was covered with a film containing the preservative and compared to controls without preservative. The samples were cultivated at 37°C and examined at frequent intervals.

Microorganism	Species
Bacteria	*Bacillus cereus*
	Pseudomonas fragi
Yeast	*Saccharomyces cereviseae*
Moulds	*Aspergillus niger*
	Penicillium roqueforti
	Rhizopus stolonifer

Table III Microorganisms used to probe the antimicrobial effect of films containing preservatives

For the bacterial contamination, there was substantial growth on and under the NPS and WPI films without PHB, whereas the films containing PHB only showed minor growth around the edges and in wrinkles (Fig. 2). The results were similar for the yeast contamination, but showed slightly worse resistance with PHB. The monoglyceride films were more resistant to growth of bacteria even without PHB and only showed minor growth around the edges of the films. With the addition of PHB the growth of bacteria was prevented totally. The yeast growth was minor for all films and was not affected by the PHB addition. The WPI and NPS films without PHB showed no inhibiting effect on mould growth whereas addition of PHB totally inhibited growth. The monoglyceride film had a good effect on mould growth even without added PHB, probably to its acidic character combined with the change in oxygen availability.

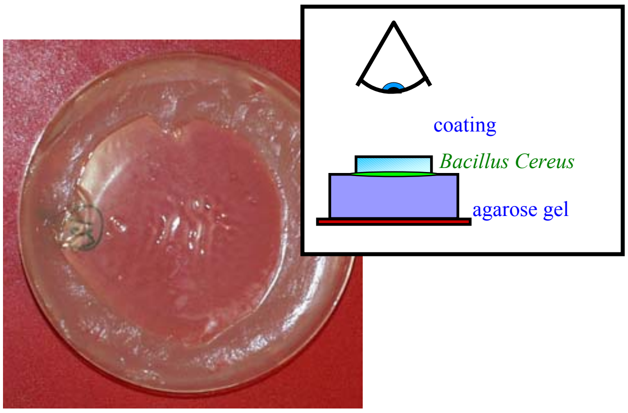

Figure 2 A whey protein film containing PHB on top of the model food. The grey substance surrounding the film is a layer of *Bacillus cereus*. Note the clear, bacteria-free area under the film.

THERMOPLASTIC PROTEIN FILMS

The solvent process is the most common technique used to prepare films based on zein proteins. This process is based on the dispersion or solubilization of the proteins in a solvent medium. The film is then obtained by a number of different separation techniques, including the solvent removal (drying). Zein films, prepared with this technique, are brittle at room conditions and plasticizers such as polyethylene glycol (PEG), glycerol, lactic acid, and fatty acids should be added to induce softness and permanent flexibility.

As for the processing of thermoplastic starch-based materials, proteins can be processed by using their thermoplastic behaviour. In this way, several products can be formed by using traditional shaping methods, such as extrusion, compression melding, thermoforming, calendaring, injection moulding. In all these operations, shear and heat is applied in order to produce a macromolecular flow. The possibility of using proteins with thermoplastic processing technologies is directly related to the existence of a glass transition temperature (Tg). Above Tg, the material should flow under an applied shear and this is possible if intermolecular interactions or cross-linkings are not present. Natural occurring polymers have been chemically modified in the past (cellulose acetate) in order to reduce the strong hydrogen bondings and to reduce the Tg of the biopolymer. In the case of zein, a reduction of Tg, necessary to avoid degradation phenomena during processing, can be achieved by using a suitable plasticizer, whose physical-chemical characteristics are optimized on the basis of the hydrophilic/hydrophobic properties of the proteins. In order to modify the structural organization and the strong intermolecular interaction of the proteins, intensive mechanical shear must be applied during heating and mixing with plasticizers.

Thermoplastic zein was prepared by using a lab internal batch mixer (Fig. 3), equipped with a monitoring system of the principal process parameters, such as temperature and torque. Usually, the mixing process is affected by several processing variables such as mixing temperature, time of mixing, and speed of rotation. The mechanical properties of the plasticized zein, used to prepare compression moulded sheets, showed that an increase of the plasticizer content resulted in materials with lower tensile strength and lower elastic modulus (Table IV). The modification of the tensile properties was not only dependent on the amount of plasticizer, but also on its compatibility with the protein and the processing conditions employed. These results were confirmed by the analysis of the glass transition temperature, measured by means of dynamic-mechanical tests. As expected, the Tg of plasticized zein was affected by both the plasticizer type and its content.

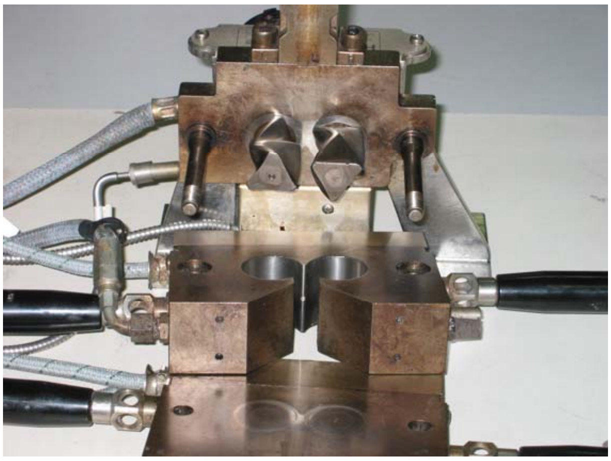

Figure 3 Mixing chamber (V=50 cm^3) utilized for the preparation of thermoplastic zein.

The hot-pressed films were transparent, strong and flexible with properties similar to the cast films, prepared with the same type of plasticizers. However, lower plasticizer content was sufficient to produce equally flexible films.

CONCLUSIONS

Polysaccharide and protein based biopolymers have the potential as coatings and biofilms to improve of quality of fruits and vegetables by preventing deterioration. They can act moisture barrier and gaseous barrier to create a modified atmosphere for shelf life extension. The edible coatings can also be used as release carriers for additives. Edible coatings containing preservatives proved to efficient in inhibiting proliferation of a surface contamination of bacteria, yeast or moulds.

Plasticizer, %w/w	Tensile strength MPa	Tensile modulus MPa	Strain at break mm/mm
Type I, 20%	17.4±5.7	918.9±126.1	0.035±0.003
Type I, 25%	3.0±0.44	165.5±9.5	0.641±0.026
Type I, 30%	1.7±0.2	80.8±19.2	0.984±0.010
Type II, 25%	4.7±1.1	217.4±10.3	0.204±0.013
Type III 25%	2.7±1.1	144.5±16.8	0.120±0.046
Type III 30%	0.7±0.2	23.9±9.0	0.752±0.240
Type IV, 25%	13.8±2.2	820.3±25.4	0.025±0.007
Type IV, 30%	11.5±1.2	770.9±50.2	0.018±0.003

Table IV Effects of type of plasticizer and its content on the tensile properties of thermoplastic zein films

Several plasticizers, with different hydrophobic/hydrophobic characteristics and molecular weight were used to investigate the thermoplasticity of zein proteins and to modify their physical-chemical and rheological properties. As for synthetic thermoplastic polymers, the use of traditional thermoplastic processing technologies requires a concurrent optimization of product and process that, in case of proteins, is complicated by the lower thermal stability that restricts the processability window.

REFERENCES

1. Stading, M., Rindlav Westling, Å. and Gatenholm, P. Humidity-induced structural transitions in amylose and amylopectin films. *Carbohydrate Polymers* **45** (2001) 209-217.
2. Arvanitoyannis, M. and Blanshard, J.M. Study of diffusion and permeation of gases in undrawn and unaxially drawn films made from potato and rice starch conditioned at different relative humidities. *Carbohydrate Polymers* **24** (1994) 1-15.
3. Mark, A.M., Roth, W.B., Mehltretter, C.L. and Rist, C.E. Oxygen permeability of amylomaize starch films. *Food Technology* **20** (1966) 75-77.
4. Rankin, J.I., Wolff, I.A., Davis, I.A. and Rist, C.E.. Permeability of amylose film to moisture vapor, selected organic vapors, and the common gases. *Industrial Engineering Chemistry* **3** (1958) 120-123.
5. Wills, R.H., McGalsson, B., Graham, D. and Joyce, D. Postharvest: An Introduction to Physiology and Handling of Fruits, Vegetables and Ornamentals, CABI and NNSW Press, Sydney, Australia (1998) pp 33-59, 144-158.
6. Ahvenainen, R. New approaches in improving the shelf life of minimally processed fruits and vegetables. *Trends in Food Science and Technology* **7** (1996) 179-186.
7. Garcia-Gimeno, R.M. and Zurera-Cosano, G. Determination of ready to eat vegetable salad shelf life. *International Journal of Food Microbiology* **36** (1997) 31-38.
8. Cisneros-Zevallos, L., Saltveit, M.E. and Krochta, J.M. Mechanism of surface white discoloration of peeled (minimally processed) carrots during storage. *Journal of Food Science* **60** (1995) 320-333.
9. Bolin, H.R. and Huxsoll, G.C. Control of minimally processed carrot (*Daucus carota*) surface discoloration caused by abrasion peeling. *Journal of Food Science* **56** (1991) 416-418.
10. Carlin, F., Nguyen-The, C., Cudennec, P. and Reich, M. Microbiological spoilage of fresh ready to use grated carrots. *Science des Aliments* **9** (1989) 371-386.
11. Merck, Microbiology Manual, Merck, Darmstadt, Germany, (1990) pp 96, 132, 168.
12. Baldwin, E.A., Nisperos-Carriedo, M.O. and Baker, R.A. Use of edible coating to preserve quality of lightly (and slightly) processed products. *CRC Critical Reviews in Food Science and Nutrition* **35** (1995) 509-524.
13. Chen, X., Campbell, G.A., Grant, L.A., Li, P. and Barth, M.M. Effect of nature seal on maintaining carotene in fresh-cut carrots. *Proceedings of the Florida State of Horticultural Society* **109** (1996) 258-259.
14. Baldwin, E.A., Nisperos-Carriedo, M.O., Chen, X. and Hagenmaier, R.D. Improving the storage life of cut apple and potato with edible coating. *Postharvest Biology and Technology* **9** (1996) 151-163.
15. Park, H.J., Chinnan, M.S. and Shewfelt, R.L. Edible corn-zein film coating to extend storage life of tomatoes. *Journal of Food Processing and Preservation* **18** (1994) 317-331.
16. Rakotonirainy, A.M., Wang, Q. and Padua G.W. Evaluation of zein films as modified atmosphere packaging for fresh broccoli. *Food Chemistry and Toxicology* **66** (2001) 1108-1111.

17. DeRose, R., Ma, O.P., Kwon, I.S., Hasnain, S.E., Klassy, R.C. and Hall, T. Characterisation of kafirin gene reveals extensive homology with zein from maize. *Plant Molecular Biology* **12** (1989) 245-256.
18. Ghaouth, A.E., Arul, J., Pannampalam, R. and Boulet, M. Chitosan coating effect on the storability and quality of fresh strawberries. *Journal of Food Science* **56** (1991) 1618-1620.
19. Zuang, R., Beuchat, L.R., Chinnan, M.S., Shewfelt, R.L. and Huang, Y.W. Inactivation of *Salmonella montevideo* on tomatoes by applying cellulose-based edible films. *Journal of Food Protection* **59** (1996) 808-812.
20. Lerdthanangkul, S. and Krochta, J.M. Edible coating effects on postharvest quality of green bell peppers. *Journal of Food Science* **61** (1996) 176-179.
21. Holmström, E. Release from edible films. Masters thesis, Chalmers University of Technology, Gothenburg, Sweden (2000).
22. Stading, M. Design of edible materials – why and how? In 'Proceedings of the 3rd International Symposium on Food Rheology and Structure', (P. Fischer, Y. Marti and E. Windhab eds.) (2003) pp 89-96.