DEVELOPING OF EDIBLE PACKAGING MATERIAL BASED ON PROTEIN FILM

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Abstract

Nowadays due to the accelerated pace of industrial development in particular in food processing the nessesety of the new kinds of packaging materials with desired functional properties (such as bacteriostatic activity for increasing the shelf life for raw materials and ready products) arised. However, the problem of using of non-degradable plastics in packaging that harms the environment remains unsolved. In this study we have attempted to develop an edible and biodegradable packaging material possessing activity against spoilage causing microrganisms of fruit and vegetable products

Keywords: microbial transglutaminase, gelatin film, strength, elasticity, bacteriostatic activity.

Introduction

In world practice different film-forming materials used for food packaging which are produced as synthetic and biogenic composites. The leadingtrend of scientific developments in this direction is the creation of edible coatings.

The edible films can be obtained from protein, polysaccharide and lipid substances. Among them, the most attractive are the edible protein-based films. They have higher barrier properties than films produced from lipids and polysaccharides. However, poor stability of the protein films to water vapor and their low mechanical strength are limited their using in food packaging. Thus, modification of protein-based films must be aimed primarily at improving the mechanical strength and barrier properties of the packaging material with respect to moisture (Bourtoom, 2009).

Chemical modification helps to achieve increasing plasticity (Park et al., 2008). The most commonly used plastisizers include various polyols (glycerine), oligosaccharides and lipids (monoglycerides, phospholipids) that are destroying the hydrogen bonds between polymer chains, make structure more fluid, thereby increasing the elasticity. However, the barrier properties of the film are reducing (Hettiarachchy, Eswaranandam, 2005); besides these agents significantly increase the hydrophilicity of the material and as a result, increase its vapor permeability.

Successful attempts of increasing the elongation at break of the films were obtained when was applied formaldehyde and ethylene glycol (Wu, Zhang, 2001), however, due to the high toxicity of these compound, they can't use in the food industry.

The most promising approach for modifying the structure of the protein components of the films is the use of enzymatic methods, and a special place among them is the use of microbial transglutaminase (mTG). TG (protein-glutamine γ-glutamyl transferase, EC 2.3.2.13) – common in the nature enzyme involved in vital biological functions. mTG catalyzes an acyl- transfer reaction between the γ-carboxyamide group of peptide - bound glutamine residues (acyl donor) and various primary amines (acyl acceptor), including ε-amino group of lysine residues. This crosslinking may be both intra- and intermolecular that in the latter case leads to an increase in molecular weight protein molecules. The reaction proceeds according to the scheme:

$$R_1\text{-Glu-CO-NH}_2 + H_2N\text{-}R_2 \rightarrow R_1\text{-Glu-CO-NH-}R_2 + NH_3$$

Catalysis involving mTG led to various changes of films properties depending on the protein used but there was an increase barrier and mechanical properties in all cases. Thus, in the case of whey protein concentrate, soy isolates (SI) and their mixtures in various ratios were used, reduction in permeability to oxygen and water vapor were observed, but the elongation at break was increased under these conditions (Su, 2007). The vapor permeability decreased when mixture of gelatin and casein were used (Chambi, 2006). The vapor permeability decreased and the polymeric structure compressed when mixture of gelatin and sodium caseinate were used (Bruno, 2008). Films obtained from fish gelatin and treated by microbial TG had shown reduced transparency and reduced elongation at break, compressive structure and good barrier properties (Yi, 2006). In cases of film material comprising various composites by the enzyme treatment also leads to an improvement of some characteristics of the obtained materials. Thus, pectin – SI films prepared using mTG, show an increase in strength characteristics, but the elasticity of the films decreases (Mariniello et al., 2003). Processing transglutaminase caused an increase and decrease in the mechanical strength of the strain. Finally, it has been found that the effectiveness of the barrier to oxygen and carbon dioxide in an enzyme – linked films markedly improved, with decreased permeability to water. These data showed promising application of transglutaminase for the regulation of the mechanical properties and films permeability containing proteins (Di Pierro et al., 2006). The processing enzyme – ovalbumin chitosan films decreases their solubility in water and increases the mechanical strength, barrier properties against gases and water vapour at the same time, and also reduces the

amount of swelling (Di Pierro, 2007).
Along with the development of biodegradable films, there has been actively working on giving them antibacterial properties using different agents: nisin (Rossi-Márquez et al., 2009), propylparaben (Chung et al., 2001), potassium sorbate (Flores et al., 2003), lysozyme (Buonocore et al., 2003).
The protein film can also function as a carrier of antimicrobial agents (Franssen et al., 2002). The ultimate goal of these modifications is to increase the retention and preservation of food raw materials and ready products.
This work proposes to use as a packaging material with preservative properties of gelatin-based film, and its influence on the characteristics of the product is described below.

Materials and Methods

A commercial preparation of mTG Activa EB with an activity of 100 units (Adjinomoto Co), glycerol (Vecton), porcine pig gelatin (LLC Norden), nisin (LLC Norden), potassium sorbate (LLC Norden) were used in the experiments.

Composition of films

In the study the composition of the film material containing gelatin and glycerine was used. There were some variants of films with different compositions at the previous stage. The film with the best organoleptic characteristics like transparency, strength, elasticity was chosen. The enzyme was applied in accordance with the recommendations of technology – supplier (0.01 g g^{-1} protein). Because protein films are very fragile plastisizers are added to the composition for giving them elasticity. Glycerine was used as plastisizer in the study. Composition of analyzed material is shown in a Table 1.

Table 1

Composition of analyzed material

Component	Test sample	Control sample
	%	%
Gelatin	4	4
Glycerine	2	2
mTG, U	4.3 10^{-3}	0
Potassium sorbate	0.1	0.1
Nisin	0.01	0.01
Water	to 100 mL	

Preparation of film

Gelatin was dissolved in water at a temperature of 55–60 °C. After that glycerin was added to the solution of gelatin. The mixture was stirred and then cooled to 25 °C. Then mTG preparation was added, mixed well and placed in an incubator for 30 minutes at 37 °C. Then the predissolved preservative mixture was added to the film-forming solution. After that the vollume of the mixture brought to 100 mL. The obtained solution (40 mL) is uniformly distributed over the surface coated with polyethylene (S=17×17 cm). These films were dried at room temperature and relative humidity of 50–60% within 18 hours.

Mechanical characteristics analisys

Before the test, the film thickness of the total area was measured at 10 points with accuracy of 1 micron. The mechanical characteristics study of the films, namely the tensile strength and elongation at break was performed on a tensile testing machine IR 5071-01S (LLC "Tochpribor Service") under standard conditions:

- distance between the clamps – 50±1 mm;
- sample size: width 15±1 mm, length – 100–150 mm;
- speed in relation to the moving clamp was 250 mm min^{-1};
- measuring range from 0 to 10 kg (100 N).

Tests were conducted in the dry state of the sample, the breaking strength was determined in the longitudinal direction of the sample.
Breaking load (Qmax) and elongation at break (Lmax) was calculated using the following formulas:

$$Q_{max} = \frac{F}{S}, \quad (1)$$

F – the applied load, S – section area of the sample

$$L_{max} = \frac{l-l_0}{l_0}, \quad (2)$$

l – final length of sample, l_0 – initial length of sample.

Bacteriostatical characteristics analisys

The films obtained in the previous paragraph were applied to grapes by immersion method. This kind of berries has been chosen because of its popularity among customers, but it is tend to rapid spoilage during storage. The initial contamination was found by the means of microbiological washout method, after which the control samples without the film and the film-forming composition coated prototypes were left on cold storage during the month (t=0–4 °C, relative humidity 60–65%). At regular intervals (3, 14, 30 days), microbiological washouts were made from them, which were cultivated in the MPA (meat-peptone agar) for 24 hours at 37 °C. Further colonies were counted using the formula:

$$X = \frac{n \times 10^m}{78,5}, \quad (3)$$

n – amount of microorganosms colonies on Petri dish, m – number of 10x dilutions (m = 4 in this study)
All washes were carried out on three samples. After washout, the sample was removed from the cold chamber.

Shrinkage of berries analisys

Berries samples coated with film and control samples without it were stored under described in previous paragraph conditions. At regular times (0, 3, 4, 10, 12, 14 days) they were weighed in triplicate. Further data processing was carried using the formula:

$$\% = \frac{m - m_i}{m} \ , \qquad (4)$$

m – initial weigh, m_i – weight of berry at the day of measurement.

Data processing

All the data were processed in the program Microsoft Office Excel 2010 (Microsoft Corp.).

Results and Discussion

Mechanical characteristics

During the experiments, data were obtained on tensile strength and elongation at break of films of various compositions. The measurement results were compared, the changes in mechanical characteristics percentages shown in Figure 1.

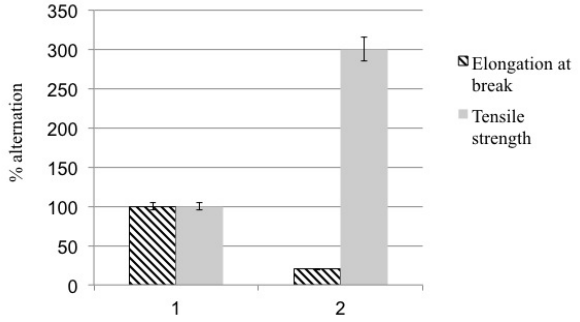

Figure 1. Mechanical characteristics changes of gelatin films causing mTG using
1 – control sample, 2– test sample with mTG

The breaking strength increases along with a decrease in elasticity, which was determined by elongation at break, in all cases of application of the enzyme. In our opinion, these changes occur because of the formation of crosslinks between the polymer chains of the protein, causing their mobility in relation to each other within the polymer network to reduce, by this reason there were decreasing in elasticity and increasing in strength.

Bacteriostatic activity

Manufactures is known to prolong the shelf life of the product by the addition of various preservatives on the surface of the fruit, so before our experiments, all berries were thoroughly washed with warm water for half an hour. Then they were dried for a day at room temperature and constant winding then the experiment was started which results are shown in Table 2 and Figure 2.

Table 2

Microorganisms amount

Day of storage	Test sample	Control sample
	CFU×cm^{-3}	CFU×cm^{-3}
0	(0.6 ±0.03)×10^4	(0.6 ±0.03)×10^4
3	(0.4 ±0.06)×10^4	(2.2 ±0.05)×10^4
14	(3.2±0.04)×10^3	(7.0±0.03)×10^4
30	(0.6 ±0.03)×10^3	(7.5 ±0.02)×10^4

Evaporation is one of the main reasons of weigh losses during storage. Some authors reported that mTG is capable to decrease water vapour permeability (Di Pierro, 2007), so we can suggest that the mTG-treated films can decrease weigh losses because of water evaporation.

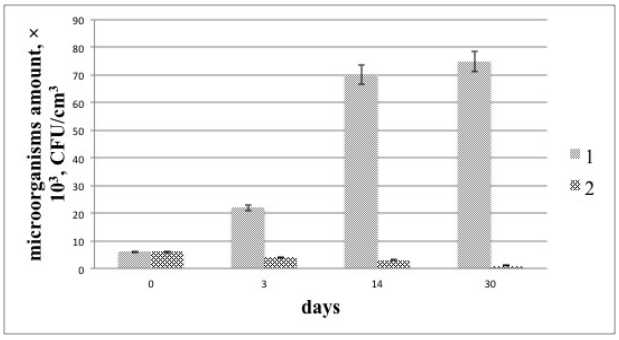

Figure 2. Microbological index changing
1 – control sample, 2– test sample with mTG

As we can see the significant decrease in microorganisms quantity is observed in case of film application. The strong tendency of microphlora reduction is notable during storage for packaged samples, so we can conclude that the coating is capable to decrease and restrain bacteria growth.

Shrinkage during storage

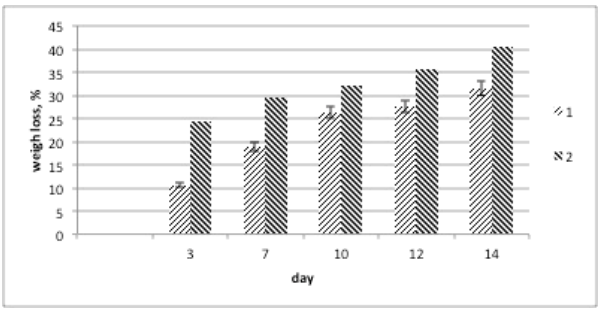

Figure 2. Weight loss during storage
1 – test sample with mTG, 2–control sample

The results obtained showed the decrease in weigh loss during the storage for samples coated in film. It can be concluded that the film could prevent shrinkage.

Conclusions

We have developed and investigated edible packaging material based on porcine gelatin with preservative properties. Because of using cross-linking agent – mirobial transglutaminase, it showed acceptable mechanical properties. At the same time, because of the preservative composition used based on potassium sorbate and nisin, it demonstrated antimicrobial action. Selected packaging material composition had no significant effect on the appearance of the packaged product, and, if desired, can be washed away from the surface of the sample.

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