

Mechanical and Permeability Properties of Milk Protein Films

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ABSTRACT

Edible films present a potential alternative for replacing plastic films used for packaging in food industry. One of the major advantages is the environmental appeal of this technology, which produces no packaging waste. Some films made with other edible materials have found commercial applications, and many more are being developed using a myriad of food based components. This paper focuses on some important characteristics of films produced with sodium and calcium caseinates. The effects of caseinate type and concentration, plasticizer concentration and pH were studied. Major parameters investigated were solubility, tensile properties, water vapour and oxygen permeabilities. Caseinate films showed high solubility at pH range 6.0-8.0 and complete insolubility at pH 3.0 and 4.0. Calcium caseinate films had a higher tensile strength and a lower % elongation at break than sodium caseinate films. There was not significant difference in water vapour and oxygen permeabilities between sodium and calcium caseinate films.

Key words: edible films; sodium caseinate films; calcium caseinate films

INTRODUCTION

Edible films are defined as thin layers of edible materials applied on food products in a such way that a selective barrier is formed against water vapour, gases and solutes transmission, providing mechanical resistance. Edible films are usually formed from a solution or dispersion of the film forming agent, followed by separation of the fluid carrier or by solidification of the film forming agent (Kester & Fennema, 1986).

The film forming agent is necessarily a long chain polymer with high cohesion force and coalescence capacity which make possible the formation of the film structures (Banker, 1966).

The degree of cohesion determines the properties such as density, porosity, permeability, flexibility and resistance of the films (Guilbert, 1986). The composition and concentration of the film forming dispersion and the film thickness define the degree of film cohesion (Guilbert, 1986). The cohesion property of a film is also determined by the chemical nature and structure of the polymer, solvent

characteristics, presence of additives and the environmental conditions during film formation (Guilbert, 1986; Kester & Fennema, 1986).

The separation of the film forming agent from the solvent has been achieved by several processes: simple and complex coacervation, thermal gelatinization and precipitation (Kester & Fennema, 1986).

Among the main additives utilized in film production are the plasticizers: non-volatile substances, with high boiling point that when added to a polymeric film, change its physical and mechanical properties. The plasticizers reduce intermolecular bonds between adjacent polymeric chains, resulting in a decrease of the cohesion and tensile forces, causing an increase in flexibility, tear resistance and water vapor and gases permeability (Banker, 1966; Guilbert, 1986).

The functional characteristics to be considered for a specific food application depend on the food product and its mode of deterioration. For instance, to protect fatty food from oxidation a film resistant to oxygen transport would be

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indicated; if the objective is to retard desiccation of fruits and vegetables, some oxygen and carbon dioxide permeability is desired to avoid anaerobic respiration (Antunes, 1996; Folegatti & Antunes, 1996).

We studied the influence of protein type and concentration, plasticizer concentration and pH on film formation. The parameters evaluated in order to optimize conditions for the application of the films as packaging materials were film solubility, mechanical resistance and water vapour and gases permeability.

MATERIALS AND METHODS

Raw materials

Sodium caseinate (NaCNT) (ALANATE 185) and calcium caseinate (CaCNT) (ALANATE 385), both from New Zealand Milk Products, were tested as film forming agents. Glycerol (Merck) was used as plasticizer.

Treatments

At a first stage, NaCNT and CaCNT were evaluated regarding the variables: protein concentration (4 and 8%), glycerol concentration (1, 2 and 3%), pH (6.5 and 9.0 for NaCNT and 6.7 and 9.0 for CaCNT), homogenization (90 and 120 sec for NaCNT and 30 sec for CaCNT), de-aeration (30, 60, 80, 90, 100 and 120 min for NaCNT and 30 min for CaCNT) and volume of dispersion applied on the plates (0.10 and 0.13 mL/cm² for NaCNT and 0.10 mL/cm² for CaCNT).

At a second stage, the variables studied for NaCNT (treatments T1, T2, T3, T4, T3B and T4B) and for CaCNT (treatments T5, T6, T7, T8, T7B and T8B) were: protein concentration (4% for T1, T2, T5 and T6 and 8% for T3, T4, T3B, T4B, T7, T8, T7B and T8B), glycerol concentration (1% for T1, T2, T3, T4, T5, T6, T7 and T8 and 3% for T3B, T4B, T7B and T8B) and pH (6.5 for T1, T3 and T3B, 6.7 for T5, T7 and T7B and 9.0 for T2, T4, T4B, T6, T8 and T8B).

Film forming procedures

NaCNT or CaCNT were dispersed in distilled water. After addition of the plasticizer agent (glycerol), homogenization was performed in a waring-blender, followed by de-aeration under vacuum. 0.1N NaOH was used to adjust the pH to 9.0. The dispersions were poured on acrylic plates (15 x 15 cm) and dried under the hood, under room conditions (temperature and relative humidity - RH).

At the second stage, the homogenization and de-aeration times of the dispersions were, respectively, 30 sec and 30 min for treatments T5, T6, T7 and T8, 60 sec and 30 min for treatments T7B and T8B, 90 sec and 60 min for treatments T1 and T2 and 120 sec and 120 min for treatments T3, T4, T3B and T4B. Disposable Petri dishes (8.7 cm diameter) were used as the physical support for film formation instead of the acrylic plates (15 x 15 cm). The applied volume of the dispersions from treatments T1 – T4B and T5 – T8B was 0.10 mL/cm².

Analyses

At the first stage, only qualitative parameters were evaluated: structure integrity, transparency, flexibility and mechanical resistance of the films.

Moisture and crude protein were determined according to procedures described by A.O.A.C. (1995).

Water solubility of the films from treatments T1-T4 and T5-T8 were qualitatively measured by the Biuret reaction (Gornall *et al.*, 1949).

The solubility of the films (from treatments T1-T4 and T5-T8) at different pH values was determined as described by Motoki *et al.* (1984) with modifications proposed by Folegatti *et al.* (1997). Ten mL of McIlvaine buffer (pHs 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0) were added to an amount of film containing 50 mg protein. The mixture was agitated in a Vortex for 1 min, followed by filtration through filter paper (Whatman # 1). One mL of filtrate was added to 4 mL of Biuret reagent (Gornall *et al.*, 1949).

After 30 min, absorbance was measured at 540 nm. The protein concentration of the samples was calculated based on a standard curve (BSA - bovine serum albumin). The results were calculated as % of soluble protein.

Films from treatments T3B, T4B, T7B and T8B had their solubility tested at different pHs. Five mL of McIlvaine buffer (pH 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0) were added to an amount of film containing 100 mg protein. The mixture was agitated in a Vortex at low speed for 15 sec, kept without agitation for 2 h, agitated again in a Vortex at high speed for 15 sec and followed by filtration through filter paper (Whatman # 1). Crude protein was measured in the total volume by semimicro-Kjeldahl's procedure (A.O.A.C., 1995). The results were calculated as % of soluble protein.

The mechanical properties of the films were measured in a Qtest equipment with load cell of 4450 N capacity, crosshead speed of 4.23×10^{-4} m/s, gage length of 5.08 cm and a sample width of 1 cm. Tensile strength and break elongation were also evaluated.

Water vapour permeability was determined in a chamber with saturated solution of NaCl to assure a relative humidity of 75% at 15°C. Film samples were utilized as barriers between the interior of recipients with desiccant material and the environment of the chamber for a 2 d period.

The measurements of oxygen transmission (treatments T1 – T4 and T5 – T8) were carried out in an Oxtran, under the following conditions: temperature 23°C, 50% relative humidity, pressure difference 98.0 kPa. Oxygen permeability was calculated by multiplying the oxygen transmission rate by film width and dividing the product by 101.3/98.0 kPa.

Statistical analysis

Data from moisture and crude protein (T1-T4B and T5-T8B), mechanical properties and water vapour permeability determinations (T3B, T4B, T7B and T8B) were analyzed according to an one-way and equal replication model and for

film solubility at different pH determinations (T1-T4B and T5-T8B), a two-factor and equal replication model was utilized.

RESULTS AND DISCUSSION

First stage

All the treatments based on sodium caseinate yielded films with good integrity, flexibility and transparency. McHugh & Krochta (1994) pointed out that the aqueous solutions of caseinates produced films with desirable integrity due to open nature of the casein molecules and their property of being able to form intermolecular crosslinks as a result of hydrogen, electrostatic and hydrophobic interactions. The films produced from 8% protein dispersions were thicker and more rigid than the films made from 4% protein dispersions. The increase in plasticizer concentration had a great influence on films flexibility. pH had no influence on film formation. Time required for homogenization and de-aeration of the dispersions were greater when the protein concentration was 8%. The application of 0.10 mL/cm² was satisfactory; when 0.13 mL/cm² were applied the films were thicker and more rigid.

Treatments based on calcium caseinate produced films with good integrity and flexibility. Films made from 8% protein were thicker and more rigid than those formed from 4% protein. The utilization of glycerol at 2 and 3 % promoted a satisfactory film flexibility. pH had influence on film transparency: at pH 6.7, the films were not completely transparent and at pH 9.0, they were more transparent but a little blurred.

Second stage

Moisture content

Films formed from sodium caseinate with 1% glycerol (T1-T4) showed an average moisture content of 13.41%. The moisture content of films formed from 8% protein was higher ($p \leq .01$) than that of films formed from 4% protein (T1<T3 and T2<T4) (Figure I). The influence of protein concentration on moisture content is

explained by water retention in the film structure through hydrogen bonds, ionic hydration and hydrophobic interaction (Banerjee & Chen, 1995). Treatments at pH 9.0 resulted in films with higher moisture content ($p \leq .01$) than films from

treatments at pH 6.5 ($T1 < T2$ and $T3 < T4$) (Figure II). As the pH gets far away from the casein isoelectric point, the water solubility of the caseinate and the water retention in film structure were increased.

Figure 1. Influence of protein concentration on moisture content of sodium and calcium caseinate films with 1% glycerol.

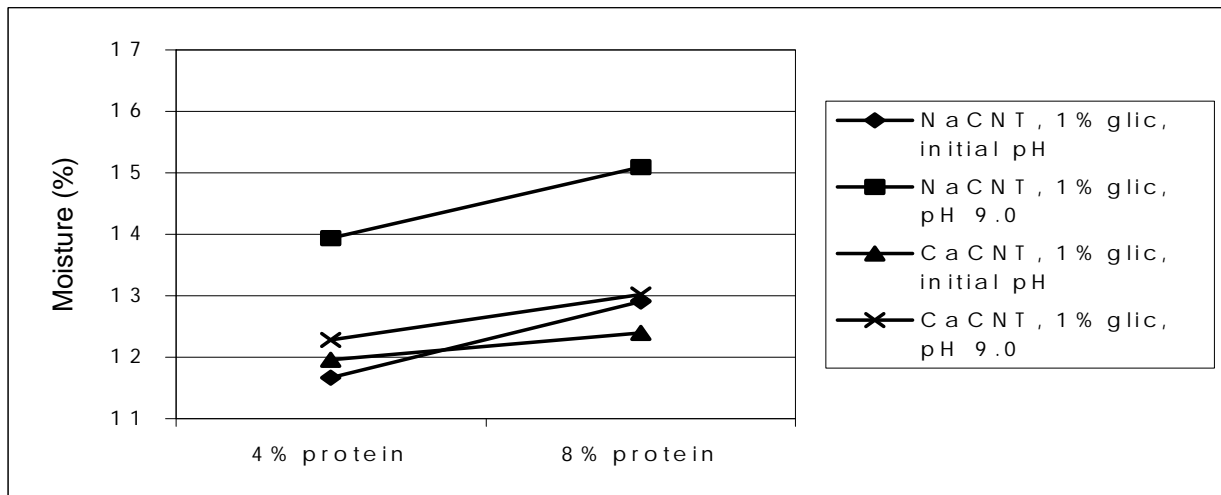
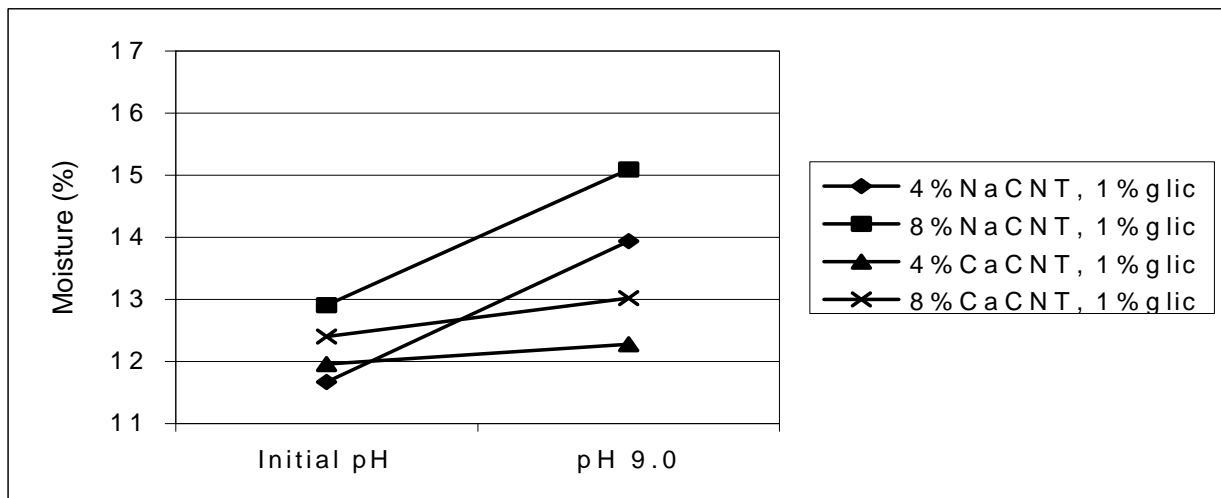


Figure 2 Influence of pH on moisture content of sodium and calcium caseinate films with 1% glycerol.



Calcium caseinate with 1% glycerol films (T5-T8) had an average moisture content of 12.41%, which was lower than the value obtained for sodium caseinate with 1% glycerol films. These results are in agreement with those reported by Banerjee & Chen (1995). Films with 8% protein at pH 9.0 showed higher moisture content ($p \leq .01$)

than those with 4% at pH 9.0 ($T6 < T8$) (Figure I) and those with 8% protein at pH 6.7 ($T7 < T8$) (Figure II).

Sodium caseinate with 3% glycerol films (T3B and T4B) had an average moisture content of 13.01%, which was not significantly different

($p \leq .05$) from the average moisture content of calcium caseinate with 3% glycerol films (T7B and T8B) - 12.70%. No significant difference ($p \leq .05$) was observed among treatments with initial pH and pH 9.0 (T3B, 12.66% = T4B, 13.36% = T7B, 12.59% = T8B, 12.80%).

Crude protein content

The crude protein content of caseinate with 1% glycerol films (T1-T4 and T5-T8) was not significantly different ($p \leq .05$) with an average value of 62.8%. There was no significant difference ($p \leq .05$) between films made from dispersions containing 4 and 8% protein. The dispersions containing a higher protein content were able to retain higher amount of water; films resulting from these treatments did not show a higher crude protein content/weight of film. There was not significant difference ($p \leq .05$) between the sodium and calcium caseinate with 1% glycerol films (T1, 59.8% = T2, 63.1% = T3, 63.5% = T4, 63.7% = T5, 62.3% = T6, 63.1% = T7, 64.6% = T8, 62.0%).

Sodium caseinate with 3% glycerol films (T3B and T4B) had an average crude protein content of 63.24%; for calcium caseinate with 3% glycerol films, the crude protein content was 62.34%. Crude protein content for sodium caseinate with 3% glycerol at pH 6.5 films was significantly higher ($p \leq .05$) than that for calcium caseinate with 3% glycerol at pH 6.7 films (T3B, 64.24% > T7B, 61.97%), and the crude protein content of sodium and calcium caseinate at pH 9.0 films did not differ ($p \leq .05$) (T4B, 62.24% = T8B, 62.70%).

Water solubility

Sodium and calcium caseinate with 1% glycerol films (T1-T4 and T5-T8) were water soluble. These results are in agreement with those reported by Motoki *et al.* (1987), Krochta *et al.* (1988) and McHugh & Krochta (1994).

Protein solubility at pH 3.0 - 8.0

Solubility of sodium caseinate with 1% glycerol films (T1-T4) in buffered solutions in the pH range 3.0-8.0 was evaluated. Treatments T1, T2 and T3 showed higher solubility ($p \leq .01$) at pH

7.0. Below and above this pH, the solubility of the films was significantly lower. McHugh & Krochta (1994) reported solubility of caseinate at pH above 5.5. Treatments T1, T2 and T3 showed lower solubility at pH 4.0, 3.0 and 5.0. The low solubility of sodium caseinate films in pH values near the casein isoelectric point was studied by Avena-Bustillos & Krochta (1993) and Krochta *et al.* (1998). The treatment of casein films at pH 4.6 would promote a modification of the molecular conformation of the protein which might favor protein-protein interactions, causing a reduction in protein solubility.

Solubility at pH 7.0 of sodium caseinate with 1% glycerol films made with 4% protein was higher than those with 8% protein ($p \leq .01$) (T1>T3 and T2>T4). Higher protein concentration gave a better structure stability to the films. Solubility (at pH 7.0) of films at pH 6.5 was higher than treatments at pH 9.0 ($p \leq .01$) (T1, 100% > T2, 6.10% and T3, 37.27% > T4, 1.75%). These results suggested that the pH adjustment to 9.0 promoted a change in the conformational structure of the protein molecules, causing the formation of films with a stable structure and, as a consequence, low solubility.

Calcium caseinate with 1% glycerol films (T5-T8) showed higher solubility at pH 5.0-7.0 and the lowest solubility was observed at pH 4.0 < 3.0 < 8.0. The influence of protein concentration and pH increase in the degree of solubility (at pH 6.0) was observed ($p \leq .01$) (T6, 17.32% > T8, 11.12%, T5, 41.90% > T6 and T7, 43.07% > T8).

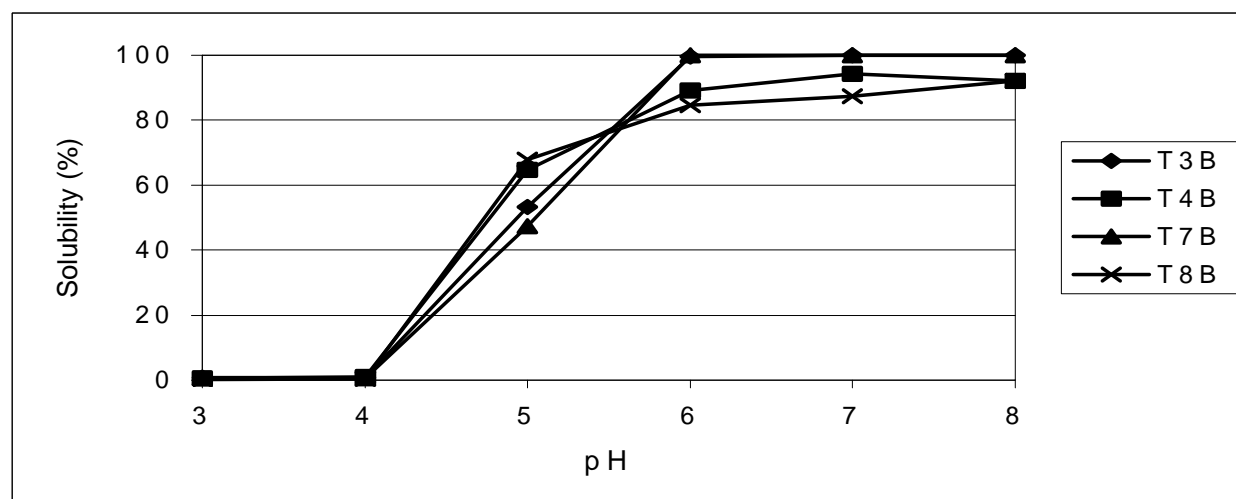
The solubility at pH 3.0-8.0 of sodium caseinate with 3% glycerol (T3B and T4B) and calcium caseinate with 3% glycerol (T7B and T8B) films is shown in Figure III. For all treatments the highest solubility ($p \leq .01$) occurred at the pH range 6.0-8.0 and the lowest solubility ($p \leq .01$) occurred at pHs 3.0 and 4.0 (pH 3.0 = pH 4.0); at pH 5.0 intermediate values of solubility were obtained. It was clearly showed the influence of solvent pH on film solubility.

Solubility in the pH range 6.0-8.0 was higher for the films at neutral pH ($p \leq .01$) than for those treated at pH 9.0 (T3B>T4B and T7B>T8B); at pH 5.0 the behavior was just the opposite; at pH 3.0 and 4.0, no significant difference was observed ($p \leq .01$) between treatments at neutral pH and pH 9.0 (T3B=T4B and T7B=T8B). Under conditions that favor the solubilization of casein structures (pH above 6.0), the treatments in which the pH was adjusted to 9.0 - possibly structural modifications happened- were less soluble than the treatments at neutral pH. However, under pH conditions in which casein structures tended to insolubilization (pH near the isoelectric point), treatments at pH 9.0 produced

films which were more soluble than those at neutral pH.

Films of sodium caseinate with 3% glycerol at pH 9.0 had a significantly higher solubility ($p \leq .01$) at pH 6.0 and 7.0 than films of calcium caseinate with 3% glycerol at pH 9.0 (T4B>T8B); sodium caseinate films with 3% glycerol at pH 6.5 showed significantly higher solubility ($p \leq .01$) at pH 5.0 than calcium caseinate films with 3% glycerol at pH 6.7 (T3B>T7B). The lower solubility of calcium caseinate films was reported by Avena-Bustillos & Krochta (1993) and it has been related with calcium effect as a crosslinking promoter in the film structure.

Figure 3. Protein solubility at pHs 3.0-8.0 of sodium and calcium caseinate films with 3% glycerol.



Tensile strength

Tensile strength of the films made from sodium caseinate with 1% glycerol (T1-T4) had an average value of 48.27 MPa. Treatments in which the protein content was 8% had a higher tensile strength than those with 4% protein (T1<T3, T2<T4). According to Motoki *et al.* (1987) the higher the protein concentration of the film, the higher would be its tensile strength. Treatment with 8% protein at pH 6.5 resulted in films with greater tensile strength than that with 8% protein at pH 9.0 (T3>T4). This result was

probably a consequence of the higher moisture content of the films in treatment at pH 9.0. Banerjee & Chen (1995) showed an inverse relation between moisture content and tensile strength of films.

The average tensile strength value for calcium caseinate with 1% glycerol films (T5-T8) - 37.23 MPa - was lower than that for sodium caseinate with 1% glycerol films. Films from treatments at pH 6.7 had a higher tensile strength than those from treatments at pH 9.0. (T5>T6

and T7>T8). These results were related to the moisture content of the films, higher in those films resulting from treatments at pH 9.0.

Sodium and calcium caseinate treatments with 1% glycerol produced films with higher tensile strengths than the treatments with 3% glycerol (T3>T3B, T4>T4B and T7>T7B). According to McHugh & Krochta (1994) the increase in glycerol concentration decreased the tensile strength of films. Our observations followed a similar trend. Calcium caseinate with 3% glycerol films had a tensile strength significantly higher ($p \leq .05$) than sodium caseinate with 3% glycerol films (T3B, 16.11 MPa < T7B, 21.64 MPa and T4B, 14.15 MPa < T8B, 21.79 MPa); this result was in agreement with those reported by Banerjee & Chen (1995). No significant difference ($p \leq .05$) was found between films with sodium and calcium caseinate with 3% glycerol at neutral pH and those at pH 9.0 (T3B=T4B and T7B=T8B).

Sodium and calcium caseinate with 1% glycerol films had higher tensile strengths than those found by other authors for low-density polyethylene (LDPE) and high-density polyethylene (HDPE), while caseinate with 3% glycerol films had lower tensile strengths than these synthetic films (LDPE: 13MPa, McHugh & Krochta, 1994; 31.2 MPa, Chen, 1995. HDPE: 26 MPa, McHugh & Krochta, 1994).

Break elongation

The break elongation of the films made from sodium caseinate with 1% glycerol (T1-T4) had an average value of 7.3%. At basic pH the films had a higher break elongation as a result of a higher moisture content ($p \leq .05$) (T3B<T4B). A positive correlation between moisture content and break elongation of protein films was reported by Banerjee & Chen (1995).

The average value of break elongation for calcium caseinate with 1% glycerol films (T5-T8) - 4.4% - was lower than the one for sodium caseinate with 1% glycerol films. Treatments with 4% protein resulted in films with a higher

break elongation than the treatments with 8% protein (T5>T7 and T6>T8).

The break elongation for sodium and calcium caseinate with 3% glycerol films was higher than that for treatments with 1% glycerol (T3<T3B, T4<T4B, T7<T7B and T8<T8B). According to McHugh & Krochta (1994), glycerol concentration has a marked influence on break elongation. Break elongation of films made from sodium caseinate with 3% glycerol at pH 9.0 was significantly higher ($p \leq .05$) than that of films treated at pH 6.5 (T3B, 17.18% < T4B, 28.73%) and than that of films made from calcium caseinate with 3% glycerol (T4B>T7B, 9.45% and T4B>T8B, 11.58%). There was no significant difference ($p \leq .05$) between films made from calcium caseinate with 3% glycerol at pH 9.0 and those at pH 6.7 (T7B=T8B).

Sodium and calcium caseinate films, both with 1 and 3% glycerol, had extremely low break elongation values as compared to LDPE (500%, McHugh & Krochta, 1994; 156%, Chen, 1995) and HDPE (300%, McHugh & Krochta, 1994) films.

Water vapour permeability

Water vapour permeability (at 15°C, 75% RH) of sodium caseinate with 1% glycerol films (T1-T4) had an average value of 4.79 (g.mm)/(m².d.kPa). Treatments with 8% protein had a higher permeability than those with 4% protein (T1<T3 and T2<T4).

The average value for water vapor permeability of calcium caseinate with 1% glycerol films (T5-T8) - 1.95 (g.mm)/(m².d.kPa) - was lower than that of sodium caseinate with 1% glycerol films. This was in agreement with the results published by Avena-Bustillos & Krochta (1993) and Banerjee & Chen (1995). Again, higher the protein content, higher was the water vapour permeability (T5<T7 and T6<T8).

Water vapour permeability of caseinate films was very high as compared to synthetic films [polystyrene: 0.44; polyethylene terephthalate: 0.09; polyvinyl chloride: 0.06; LDPE: 0.05; polypropylene: 0.03; HDPE: 0.01;

polyvinylidene chloride: 0.001 (g.mm)/(m².d.kPa) at 25°C, 90-100% RH (Kester & Fennema, 1986) and HDPE: 0.02 (g.mm)/(m².d.kPa) at 38°C, 90% RH (McHugh & Krochta, 1994)].

Calcium caseinate with 1% glycerol films had lower water vapour permeability than cellulose acetate films [2.87 (g.mm)/(m².d.kPa) at 25°C, 90-100% RH]; both sodium and calcium caseinate with 1% glycerol films had lower water vapor permeability than cellophane films [7.27 (g.mm)/(m².d.kPa) at 38°C, 90% RH (McHugh & Krochta, 1994)].

Oxygen permeability

The average value for oxygen permeability of films made from sodium and calcium caseinate with 1% glycerol (T1-T4 and T5-T8) were very close - 10.42 and 10.52 (cm³.µm)/(m².d.kPa) at 23°C, 50% RH, respectively.

Sodium and calcium caseinate with 1% glycerol films had lower oxygen permeability than LDPE, HDPE [respectively 1870 and 427 (cm³.µm)/(m².d.kPa) at 23°C, 50% RH], cellophane, ethylene vinyl alcohol [respectively 252 and 12 (cm³.µm)/(m².d.kPa) at 23°C, 95% RH] and polyester [17.3 (cm³.µm)/(m².d.kPa) at 23°C, 0% RH].

Several attributes could be related to caseinate films. The present concern with environmental pollution represents an appeal for the development and application of biodegradable packaging materials. These films exhibited satisfactory tensile strength and a low oxygen permeability, however, they showed a high permeability for water vapour.

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RESUMO

Filmes comestíveis representam uma alternativa potencial para a substituição de filmes plásticos empregados em embalagem de alimentos. Uma de suas maiores vantagens relaciona-se com o problema de poluição ambiental por não gerar material residual. Filmes elaborados com outros materiais comestíveis não protéicos têm encontrado aplicação comercial e muitos outros ainda estão sendo pesquisados, usando uma grande variedade de componentes alimentares. Este trabalho estuda algumas características importantes dos filmes produzidos com caseinatos de sódio e de cálcio. Os efeitos do tipo e concentração de caseinato, concentração de plastificante e do pH são investigados. Os parâmetros pesquisados foram a solubilidade, propriedades de tração e permeabilidade a vapor de água e oxigênio. Filmes de caseinato apresentaram alta solubilidade na faixa de pH entre 6.0 e 8.0 e completa insolubilidade em pH 3.0 e 4.0. Maior resistência à tração e menor percentagem de alongamento no ponto de ruptura foram observados para os filmes de caseinato de cálcio, comparados aos de caseinato de sódio. Não houve diferença significativa entre os filmes de caseinato de sódio e de cálcio, no que se refere à permeabilidade a vapor de água e oxigênio.

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