

Effect of the Maillard reaction on properties of casein and casein films

Juliana Cordeiro Cardoso · Ricardo Luiz C. Albuquerque Jr. · Francine Ferreira Padilha · Felipe Oliveira Bittencourt · Osvaldo de Freitas · Paula Santos Nunes · Newton L. Pereira · Maria José Vieira Fonseca · Adriano Antunes S. Araújo

Received: 30 March 2010 / Accepted: 7 September 2010 / Published online: 29 September 2010
© Akadémiai Kiadó, Budapest, Hungary 2010

Abstract The use of biodegradable natural polymers has increased due to the over-solid packaging waste. In this study, a chemical modification of the casein molecule was performed by Maillard reaction, and the modified polymer was evaluated by polyacrylamide gel electrophoresis (PAGE), thermogravimetry/derivative thermogravimetry (TG/DTG), FT-IR, and $^1\text{H-NMR}$ spectroscopy. Subsequently, films based on the modified casein were obtained and characterized by mechanical analysis, water vapor transmission, and erosion behavior. The PAGE results suggested an increase of molecular mass of the modified polymer, and FT-IR spectroscopy data indicated inclusion of C–OH groups into this molecule. The TG/DTG curves of modified casein presented a different thermal decomposition profile compared to the individual compounds. Mechanical tests showed that the chemical modification of the casein molecules provided higher elongation rates (45.5%) to the films, suggesting higher plasticity, than the original molecules (13.4%). The modified casein films presented higher permeability ($0.505 \pm 0.006 \mu\text{g/h mm}^3$) than the original polymer ($0.387 \pm 0.006 \mu\text{g/h mm}^3$) films

at 90% relative humidity (RH). In pH 1.2, modified casein films presented higher erosion rates ($32.690 \pm 0.692\%$) than casein films ($19.910 \pm 2.083\%$) after 8 h, suggesting an increased sensibility for erosion of the modified casein films in acid environment. In water (pH 7.0), the films erosion profiles were similar. Those findings indicate that the modification of molecule by Maillard reaction provided films more plastic, hydrophilic, and sensitive to erosion in acid environment, suggesting that a new polymer with changed properties was founded.

Keywords Biomaterials · Films · Mechanical properties · Thermal analysis · Water-soluble polymers

Introduction

Development of biodegradable packaging material to replace synthetic polymers is an approach used to reduce environmental problems. Aqueous polymers dispersions, like polysaccharides and proteins, have been studied to improve problems related with use of organic solvents [1–3].

The use of natural hydrophilic polymers has received considerable attention, especially from the viewpoint of cost, environmental pollution, safety, and biocompatibility [3–5]. Proteins and polysaccharides provide good mechanical and barriers properties. Various proteins are used for production of films and coatings due to their excellent nutritional value and functional properties. This might explain the increasing interest for using commercially available proteins to prepare biomaterials, especially films [4, 6, 7].

Casein has been studied as biomaterial, including film-forming capacity [8], matrix systems [9], coating polymer [10], and microparticles [11]. Modifications in the protein

J. C. Cardoso (✉) · R. L. C. Albuquerque Jr. · F. F. Padilha · F. O. Bittencourt
Laboratório de Biomateriais, Instituto de Tecnologia e Pesquisa/
Universidade Tiradentes, Av. Murilo Dantas, 300, Aracaju,
Sergipe 49032-490, Brazil

O. de Freitas · N. L. Pereira · M. J. V. Fonseca
Departamento de Ciências Farmacêuticas de Ribeirão Preto,
FCFRP - Universidade de São Paulo, Ribeirão Preto, Brazil

P. S. Nunes · A. A. S. Araújo
Departamento de Fisiologia, Universidade Federal de Sergipe,
Av. Marechal Rondon, s/n, Cidade Universitária, São Cristóvão,
Sergipe 49100-000, Brazil
e-mail: adriasa2001@yahoo.com.br

structure can lead changes in its characteristics. In previous research, we have studied the inclusion of acetyl groups in the casein structure [9]. The introduction of polysaccharides into specific groups of protein structure seems to be promising [12]. The reaction is known as Maillard reaction, and it occurs between amino groups and reducing carbohydrates. The final Maillard reaction consists on condensation of amino compounds and sugar fragments into polymerized protein and brown pigments, called melanoidins [12–14]. This reaction forms products with interesting characteristics, such as, flavor, antioxidant activity [14–16], antimutagenic and anticarcinogenic, antibacterial compounds [17], and higher emulsifier capacity [18–20].

The objectives of this study were twofold. First, to obtain modified casein by Maillard reaction and to evaluate this modification by polyacrylamide gel electrophoresis (PAGE), thermogravimetry (TG/DTG), FT-IR, and $^1\text{H-NMR}$ spectroscopy. Second, to prepare free films with modified casein and to characterize the films by mechanical analysis, water vapor transmission rates (WVTRs), and erosion tests.

Materials and methods

Materials

Bovine casein (biological grade Inlab[®], Brazil) aqueous dispersion 5% (w/v) at pH 7.8 and maltodextrin 10% (w/v) were mixed and heated at 90 °C for a period of 8 h.

Modification of casein (Maillard reaction)

Samples were collected during reaction (0, 2, 4, 6 and 8 h), and its kinetics evaluated by PAGE. The obtained mixture was precipitated using HCl 7 mol/L (pH 3.7), washed with HCl 0.001 mol/L in order to remove the sugar excess and dried at 40 °C for 5 h. Two controls were prepared in the same conditions (90 °C/8 h), one with only casein and other with only the polysaccharide. The powder was evaluated by thermogravimetry/derivative thermogravimetry (TG/DTG), infrared spectroscopy (FTIR), and proton nuclear magnetic resonance ($^1\text{H-NMR}$).

Polyacrylamide gel electrophoresis studies

The samples were collected during the reaction in followed times: when the dispersion casein–maltodextrin was at room temperature (RT), at time 0 (90 °C), at 2, 4, 6 and 8 h of reaction. They were dialyzed against water before PAGE studies in order to eliminate sugar excess.

These samples were mixed with a pH 8.0/10 mmol/L Tris–HCl solution of SDS (2.5 wt%), EDTA 1 mmol/L and

β -mercaptoethanol (5 wt%). The mixture was heated in a boiling water for 5 min and mixed with bromophenol blue (0.01 wt%). The final concentration was around 3 mg of protein per mL. They were subjected to the electrophoresis at 250 V, 10 mA, and 15 °C using a plate of a 8–25% gradient poly(acrylamide) gel (Pharmacia, Phast-Gel Gradient 8–25). The protein bands were stained by Coomassie brilliant blue R-250.

FT-IR and $^1\text{H-NMR}$ spectroscopy

The infrared absorption data of casein, modified casein, and maltodextrin were obtained in the range 4,000–400 cm^{-1} in KBr pellets using a Nicolet spectrophotometer model Protege 460 FT-IR at RT.

The proton nuclear magnetic resonance ($^1\text{H-NMR}$) spectra were obtained for each sample by dissolving in DMSO. Spectra were taken at 300 MHz using a BRUKER DPX 300 spectrometer.

Thermogravimetry/derivative thermogravimetry

Thermogravimetry/derivative thermogravimetry analysis of casein and modified casein was obtained in a thermobalance model TGA-50 (Shimadzu) in the temperature range from 25 to 600 °C, using platinum crucibles with ~ 3 mg of samples, under dynamic nitrogen atmosphere (50 mL min^{-1}) and heating rate of 5 °C min^{-1} . Thermogravimetric system was calibrated using a $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$ standard substance in conformity to ASTM pattern.

Films preparation

Casein or modified casein 3% (w/v) was dispersed in water and pH adjusted to 9.0 using NaOH 2.5% (w/v) solution. Glycerin was also added in ratio of 20% (w/w) of polymer as plasticizer. About 20 mL of dispersion were then cast into round polystyrene molds of 7.5 cm in diameter and dried at 40 °C during 6 h. The films were peeled off the mold and the thickness measured ($100 \pm 10 \mu\text{m}$).

Mechanical characterization: tensile testing

The mechanical analysis was carried out in a tensile strength apparatus. The mechanical test (charging cell) measures continuously the forces developed in a sample when elongated at a constant rate. Samples of films were cut into strips of 40 mm \times 10 mm. Each strip was measured at five points with a micrometer (Mitutoyo digimatic micrometer) to ensure the thickness of $100 \pm 10 \mu\text{m}$ and examined for defects such as air bubbles which could interfere with the characterization. Each experiment was carried out at RT, and 15 replicates were performed.

The measure speed used was 0.1268 mm s^{-1} and the initial gauge length was 13 mm. The calculations were made by:

$$\sigma = F/A,$$

where, σ is tensile strain in Newton mm^{-2} , F is force in Newton, and A is cross-section area in mm^2 .

Water vapor transmission rates

The permeability of casein and modified casein films was determined by water vapor loss in a gravimetric cup film sealed method under known RH given by saturated solutions in contact with non dissolved salt (ZnSO_4 90%RH or NaBr 58%RH) placed in a desiccator containing silica in a dehumidified room [21]. Each experiment was performed with eight replicates during 48 h. The permeability was calculated by:

$$\text{WVT} = w/A \cdot h,$$

where WVT is water vapor transmission in mg mm^{-3} , w is lost mass in mg, A is film area in mm^2 , and h is film thickness in mm.

$$P = \text{WVT}/t,$$

where P is permeability in $\text{mg mm}^{-3} \text{ h}$ and t is time in hour.

Erosion test

The percentage of erosion was evaluated by a gravimetric method using a SOTAX AT7 dissolution tester. Into wire mesh baskets 500 mg of dry films was placed and then weighed accurately. At predetermined intervals a basket containing the remnants of films was removed and dried at 60°C during 24 h and stored in a desiccator at RT to be weighed and the percentage of erosion calculated. The experiment was carried out using triplicates.

Results and discussion

Evaluation of product

During the reaction, a brown color was developed in the dispersion. In order to avoid bias, controls groups were made to certify that the appearing color was not a result from caramelization or denaturation. No color has appeared in the controls dispersions. It can support that the browning color was related to Maillard reaction, once this color is characteristic of the Maillard products. The investigations on heat protein–sugar mixtures revealed that the browning color is accompanied by polymerization of the protein [12, 14].

Electrophoresis results showed a molecular mass increase of the new polymer when compared to the original protein. Figure 1 displays the simultaneous disappearance of original protein band while taking place a new polymer band, in increasing times. Depending on the molecular mass, the colored compounds may be divided into two classes: low molecular mass colored compounds ($<1,000 \text{ Da}$) and melanoidins with masses up to $100,000 \text{ Da}$. The PAGE results indicated a chemical change during the reaction with an increase of molecular mass higher than $100,000 \text{ Da}$. There were polydispersed bands at the top of the gel, suggesting the formation of higher molecular mass materials. Studies of SDS-PAGE patterns of the casein–polysaccharide conjugates indicated the covalent linkage between protein and the polysaccharides [18, 19].

The spectroscopic analysis of polymeric molecules, including proteins, is complex due to the molecular vibrations arising from numerous atoms. Nine characteristics group frequencies arise from the peptide linkages [22]. The most distinctive spectral features for proteins are the strong amide I and II bands centered approximately between $1,650$ and $1,540 \text{ cm}^{-1}$, respectively [23]. For carbohydrates, a series of overlapping peaks located in the region of $1,180$ – 953 cm^{-1} results from vibration modes such as the stretching of C–C and C–O and the bending mode of C–H bonds [16]. These are often referred to as the “saccharide” bands, and are the most intense bands in the mid-infrared spectrum [24]. These absorptions are weak in the spectra of most proteins [23, 25].

The FTIR spectra of casein (A), modified casein (B), and maltodextrin (C) are shown in Fig. 2a–c, respectively. The modification could not be observed using the amide I

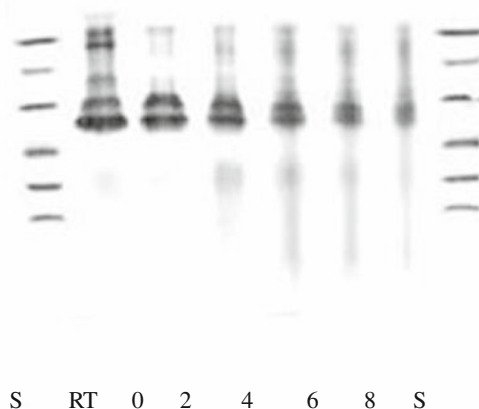
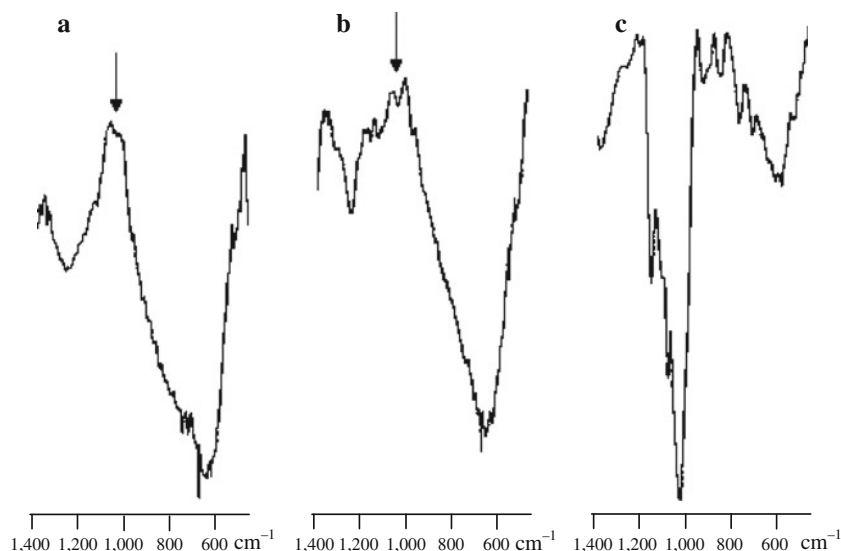


Fig. 1 PhastGel gradient 8–25 (SDS-PAGE). Samples: standard (S); casein + polysaccharide at room temperature (RT); collect dispersion at 0 h of reaction (0); 2 h (2); 4 h (4); 6 h (6); 8 h (8); standard (S)

Fig. 2 The infrared spectra of casein (a); modified casein (b); maltodextrin (c)



band, but were observed C–OH inclusions from 1,015 to 1,200 cm^{-1} region in modified casein spectrum, absent in original casein and very intense in the maltodextrin spectrum (Fig. 2). The introduction of C–OH groups in the protein may increase the hydrophilicity of the system.

In addition, $^1\text{H-NMR}$ spectroscopy analysis also indicated the occurrence of the polymer modification. In modified casein, $^1\text{H-NMR}$ spectrum could be noted some signals between 6.2 and 5.7 ppm, absent in casein spectrum and present in maltodextrin spectrum (Fig. 3).

The TG/DTG curves of pure casein show that decomposition takes place at various stages. The first decomposition may be due to loss of water (either adsorbed or bound) ($\Delta m_1 = 3.77\%$ and $\text{DTG}_{\text{peak}} = 86.9\text{ }^\circ\text{C}$). The other two events obtained above 300 $^\circ\text{C}$ are due to peptide degradation ($\Delta m_2 = 2.88\%$ and $\text{DTG}_{\text{peak}} = 243\text{ }^\circ\text{C}$; $\Delta m_3 = 51.6\%$ and $\text{DTG}_{\text{peak}} = 323\text{ }^\circ\text{C}$). In this study, TG curves of casein showed an event of fast mass loss occurring in the range of 230–360 $^\circ\text{C}$ (Fig. 4A), as well as further slow mass loss in the range of 360–600 $^\circ\text{C}$, which is usually attributed to the elimination of carbonaceous material. In general, the degradation of casein system takes place in four stages [26].

However, in the case of modified casein, it took place in five stages. The amount of polymer degraded at 100 $^\circ\text{C}$ for modified casein was 5.53% ($\text{DTG}_{\text{peak}} = 66.6\text{ }^\circ\text{C}$). At 500 $^\circ\text{C}$, the degraded amounts for the above mentioned modified were 8.7% ($\text{DTG}_{\text{peak}} = 213\text{ }^\circ\text{C}$), 37.7% ($\text{DTG}_{\text{peak}} = 315\text{ }^\circ\text{C}$), and 19.4% ($\text{DTG}_{\text{peak}} = 464\text{ }^\circ\text{C}$), respectively. These results suggest that modified casein is more stable when compared to other casein systems.

Evaluation of free films

The modified casein films showed higher plasticity than the original polymer with 20% of glycerin (w/w). The films from casein and modified casein containing 20% of glycerin showed significant differences in their mechanical profiles (Fig. 5). The elongation of casein films was about 13.4%, while the elongation of modified casein films was 42.5%. The films characterization by MA showed a film more plastic with the modified polymer whereas films with casein were more brittle in the same plasticizer conditions (Fig. 5). The rigid-chain polymers properties are known to depend on their molecular and supermolecular structures. Polymers having fibrillar structures display typical polymeric properties, while polymers having globular structures lose these properties partially or completely. The modification possibly changed the quaternary structure of polymer and its interactions interchains. Studied the relationship between triple-helix content and mechanical properties of gelatin films [27]. The mechanical properties of films containing macromolecules of different conformations respond in different ways to changes in relative air humidity.

The modified casein films presented higher permeability than the original polymer films at 90% RH (Table 1). No difference in permeability was noted at 58% RH. It suggested that, in this RH, the difference in the WVTR for the samples was not possible to detect by gravimetric method. The difference of permeability between the films may be related to an increase of hydrophilicity of the modified polymer.

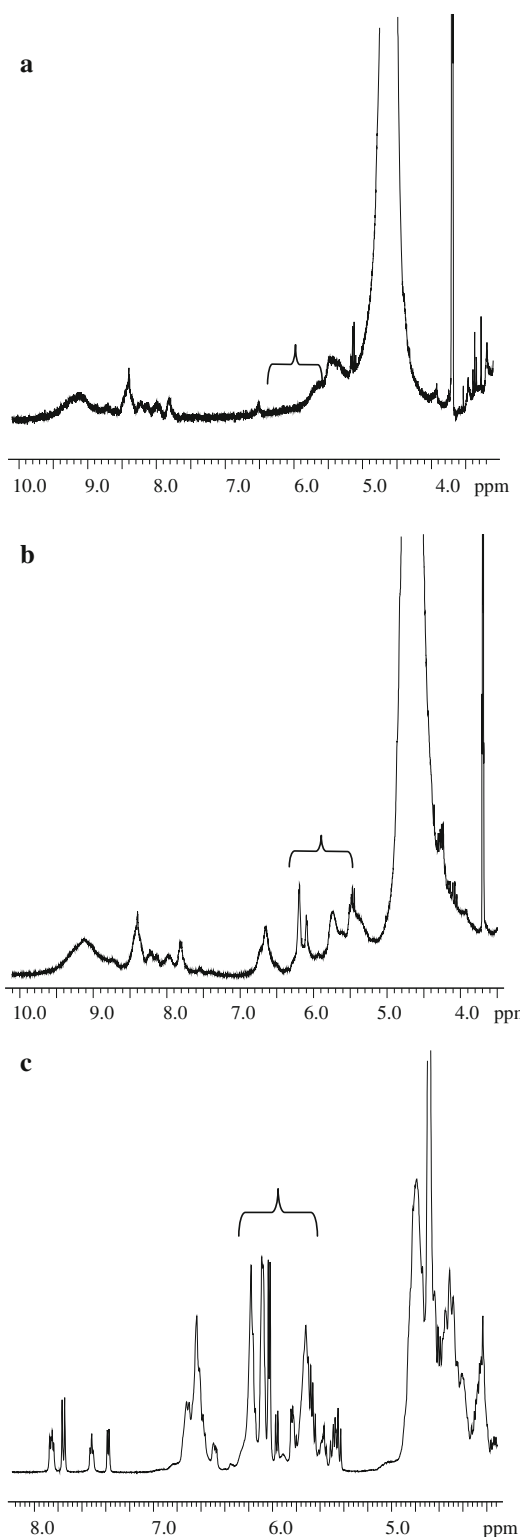


Fig. 3 The $^1\text{H-NMR}$ spectra of casein (a); modified casein (b); maltodextrin (c)

Some chemical modifications in this protein structure may also change its physicochemical characteristics, such as erosion behavior. This characteristic is important for

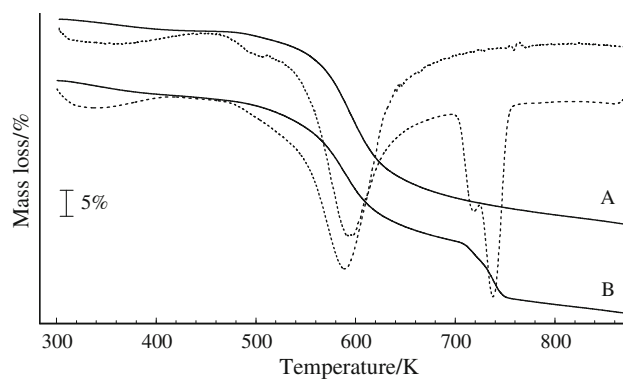


Fig. 4 TG curve (solid line) and DTG curve (dot line) of (A) casein and (B) modified casein obtained in dynamic nitrogen atmosphere (50 mL min^{-1}) and rate heating $5 \text{ }^\circ\text{C min}^{-1}$

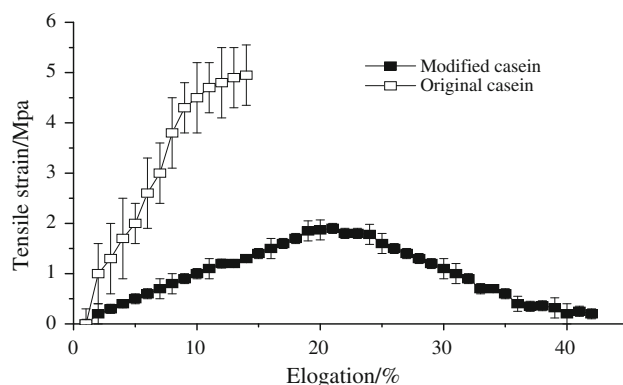


Fig. 5 Tensile strength profile of casein and modified casein films with 20% of glycerin

Table 1 Permeability of casein and modified casein films with 20% of glycerin at different relative humidity and the percentage of erosion of films in aqueous and acid environment after 8 h

| Analysis | CAS | CASGLI |
|--|--------------------|--------------------|
| Permeability/microgram/h mm^3 | | |
| 58% RH | 0.134 ± 0.001 | 0.129 ± 0.001 |
| 90% RH | 0.387 ± 0.006 | 0.505 ± 0.006 |
| Erosion/% | | |
| pH 1.2 | 19.910 ± 2.083 | 32.690 ± 0.692 |
| pH 7.0 | 98.520 ± 6.873 | 99.150 ± 1.833 |

biodegradable polymers as long as hydrophilic polymers like casein on contact with an aqueous environment gradually begin to hydrate forming a gelatinous swollen mass. Simultaneously, the polymer undergoes a relaxation process resulting in slow direct erosion of the hydrated polymer, which contributes to the drug release rate [28, 29]. The erosion results showed that in water both polymers presented the same behavior, losing 100% of mass in 8 h. In acid environment, the behaviors were different, as long

as modified casein films presented 32% of erosion and casein films 20% after 8 h, suggesting an increased sensibility for erosion in the modified casein film in acid environment (Table 1).

Conclusions

In this paper, casein–maltodextrin conjugate was prepared using Maillard. The above-mentioned studies show the effect of cross-linking of casein on its thermal stability. Protein–polysaccharide cross-linking was demonstrated using an electrophoretic method. Those findings strongly suggest the modification of molecule and its characteristics. The films with modified casein presented to be more plastic. The modified casein films showed different profiles of WVTRs and erosion behavior in acid environment suggesting that a new polymer with changed properties was founded.

Acknowledgements We would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico/CNPq/Brazil and Fundação de Amparo à Pesquisa do Estado de Sergipe/FAPITEC-SE for the financial support.

References

- Kim J-H, Kim Y-S, Park K, Kang E, Lee S, Nam HY, Kim K, Park JH, Chi DY, Park R-W, Kim I-S, Choi K, Kwon IC. Self-assembled glycol chitosan nanoparticles for the sustained and prolonged delivery of antiangiogenic small peptide drugs in cancer therapy. *Biomaterials*. 2008;29:1920–30.
- Shi P, Li Y, Zhang L. Fabrication and property of chitosan film carrying ethyl cellulose microspheres. *Carbohydr Polym*. 2008;72:490–9.
- Sothornvit R, Pitak N. Oxygen permeability and mechanical properties of banana films. *Food Res Int*. 2007;40:365–70.
- Dong A, Prestrelski S, Allison SD, Carpenter JF. Basic study of corn protein, zein, as a biomaterial in tissue engineering, surface morphology and biocompatibility. *J Pharm Sci*. 2006;84:415–21.
- López CR, Bodmeier RJ. Mechanical, water uptake and permeability properties of crosslinked chitosan glutamate and alginate films. *J Control Release*. 1997;44:215–25.
- Fabra MJ, Talens P, Chiralt A. Tensile properties and water vapor permeability of sodium caseinate films containing oleic acid–beeswax mixtures. *J Food Eng*. 2008;85:393–400.
- Nunes PS, Bezerra MS, Costa LP, Cardoso JC, Albuquerque RLC Jr, Rodrigues MO, Barin GB, Silva FA, Araújo AAS. Thermal characterization of usnic acid/collagen-based films. *J Therm Anal Calorim*. 2010;99:1011–4.
- Chambi H, Grosso C. Edible films produced with gelatin and casein cross-linked with transglutaminase. *Food Res Int*. 2006;39(4):458–66.
- Hernandes JMM, Freitas O, Pereira NL. The potential use of casein and modified casein as matrix delivery systems. *Boll Chim Farm*. 1998;137:362–6.
- Diak A, Bani-Jaber O, Amro A, Jones B, Andrews D. The manufacture and characterization of casein films as novel tablet coatings. *Food Bioprod Process*. 2007;85(3):284–90.
- Baranuskienė R, Venskutonis PR, Dewettinck D, Verhé R. Properties of oregano (*Origanum vulgare* L.), citronella (*Cymbopogon nardus* G.) and marjoram (*Majorana hortensis* L.) flavors encapsulated into milk protein-based matrices. *Food Res Int*. 2006;39(4):413–25.
- Ajandouz EH, Desseaux V, Tazi S, Puigserver A. Effects of temperature and pH on the kinetics of caramelisation, protein cross-linking and Maillard reactions in aqueous model systems. *Food Chem*. 2008;107(3):1244–52.
- Brands CMJ, van Boekel MAJS. Kinetic modelling of reactions in heated disaccharide–casein systems. *Food Chem*. 2003;83(1):13–26.
- Liu S-C, Yang D-J, Jin S-Y, Hsu C-H, Chen S-L. Kinetics of color development, pH decreasing, and anti-oxidative activity reduction of Maillard reaction in galactose/glycine model systems. *Food Chem*. 2008;108(2):533–41.
- Lertittikul W, Benjakul S, Tanaka M. Characteristics and anti-oxidative activity of Maillard reaction products from a porcine plasma protein–glucose model system as influenced by Ph. *Food Chem*. 2007;100(2):669–77.
- Gu F-L, Kim JM, Abbas S, Zhang X-M, Xia S-Q, Chen Z-X. Structure and antioxidant activity of high molecular mass Maillard reaction products from casein–glucose. *Food Chem*. 2010;120:505–11.
- Van Boekel MAJS. Effect of heating on Maillard reactions in milk. *Food Chem*. 1998;62:403–14.
- Kato A, Mifuru R, Matsudomi N, Kobayashi K. Functional casein–polysaccharide conjugates prepared by controlled dry heating. *Biosci Biotech Biochem*. 1992;56(4):567–71.
- Wooster TJ, Augustin MAJ. β -Lactoglobulin–dextran Maillard conjugates: their effect on interfacial thickness and emulsion stability. *Colloid Interface Sci*. 2006;303(2):564–72.
- Shepherd R, Robertson A, Ofman D. Dairy glycoconjugate emulsifiers: casein–maltodextrins. *Food Hydrocolloids*. 2000;14(4):281–6.
- Sprockel OL, Prapaitrakul W, Shivanand PJ. Permeability of cellulose polymers: water vapor transmission rates. *Pharm Pharmacol*. 1990;42:152–7.
- Dong J, Sun Q, Wang J-Y. Infrared spectroscopic studies of liophilization- and temperature-induced protein aggregation. *Biomaterials*. 2004;25(19):4691–7.
- Oliver CM, Kher A, McNaughton D, Augustin MA. Use of FTIR and mass spectrometry for characterization of glycosylated caseins. *J Dairy Res*. 2009;76:105–10.
- Cael JJ, Koenig JL. Infrared and Raman spectroscopy. *Carbohydr Res*. 1974;32:79–91.
- Cooper EA, Knutson K. Physical methods to characterize pharmaceutical proteins. New York: Plenum Press; 1995.
- Somanathan N, Subramanian V, Mandal AB. Thermal stability of modified caseins. *Thermochim Acta*. 1997;302:47–52.
- Bigi A, Panzavolta S, Rubini K. Relationship between triple-helix content and mechanical properties of gelatin films. *Biomaterials*. 2004;25(25):5675–80.
- Gubbins RH, O'Driscoll CM, Corrigan OI. The effects of casein on diclofenac release from hydroxypropylmethylcellulose (HPMC) compacts. *Int J Pharm*. 2003;260(1):69–76.
- Sujja-Areevath J, Munday DL, Cox PJ, Khan KA. Relationship between swelling, erosion and drug release in hydrophilic natural gum mini-matrix formulations. *Eur J Pharm Sci*. 1998;6:207–17.