Film Formation and Surface Properties of Enzymatically Crosslinked Casein Films

Helka Juvonen,[1] Maria Smolander,[2] Harry Boer,[2] Jaakko Pere,[2] Johanna Buchert,[2] Jouko Peltonen[1]

[1]*Laboratory of Paper Coating and Converting, Center for Functional Materials, Åbo Akademi University, Porthaninkatu 3, FI-20500 Turku, Finland*
[2]*VTT Technical Research Centre of Finland, FI-02044 VTT, Finland*

Received 16 April 2010; accepted 11 June 2010
DOI 10.1002/app.32943
Published online 27 August 2010 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: The use of renewable materials as barrier material is currently intensively investigated. Biopolymers such as polysaccharides, lipids, and proteins have been studied as barrier materials. Protein-based films often possess good gas barrier properties, but because of their hydrophilic nature the gas barrier properties are sensitive to humidity. The improvement of the properties of sodium caseinate barrier films in potential packaging applications was studied by investigating the effects of enzymatic treatment and plasticizer on the film properties. Oxidoreductases *Trametes hirsuta* laccase (ThL) and *Trichoderma reesei* tyrosinase (TrTyr) were compared with transglutaminase for crosslinking of the sodium caseinate molecules in the films and coatings. All of the studied enzymes were able to crosslink sodium caseinate. Film solubility tests, protein electrophoresis, contact angle measurements, and atomic force microscopy studies showed that TrTyr treatment results in sodium caseinate films and coatings with better overall properties compared to treatment with ThL. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 119: 2205–2213, 2011

Key words: enzymes; crosslinking; proteins; films; atomic force microscopy (AFM)

INTRODUCTION

The interest to use biopolymer-based packaging materials to replace petroleum-based raw materials is constantly increasing. Biopolymer-based barrier films and coatings used in packaging materials may improve the quality and extend the shelf life of products. Films made of biomass-derived natural polymers such as polysaccharides or proteins often possess good gas barrier properties. However, many natural biopolymers are hydrophilic by nature and their gas permeability may increase manifold with increasing humidity.[1–7]

The utilization of the polysaccharide and protein films as barrier materials is based on their ability to decrease gas and solute transport or oil and fat migration. Protein films tend to have lower oxygen permeability than polysaccharide films, because of their lower free volume and higher cohesive energy density caused by higher polarity and nonring structure of the proteins.[8] The use of bovine milk proteins such as casein and whey protein has been studied extensively as barrier materials.[4,5] In addition, zein, gluten, gelatin, and soy protein have been investigated extensively as barrier materials.[9–13] In all cases, it is believed that the protein molecules should have open or extended configuration to allow intermolecular interactions important for film formation.[14]

Casein films have been prepared from different types of caseins.[15–17] Compared with gelatin- and albumin-containing films, casein films have been shown to have a lower water vapor transmission rate, water gain at different humidity conditions, and higher tensile strength.[18] The chemical composition and macromolecular structure of a polymer affect mass transport within polymer films. A combination of low diffusion and low solubility coefficient of the permeant in the bulk polymer of the film results in good barrier properties.[19] It has been shown that increased crystallinity, density, orientation, or crosslinking impair the permeability of the film and improve the barrier properties.[4,8,19–22] The mass transport can be decreased by crosslinking of polymer chains by different crosslinking enzymes.[8,19,23–25] Similarly, mechanical properties of protein films can be improved using several approaches, including physical, chemical, and enzymatic methods.[25–29]

Processing parameters such as temperature, solvent, solvent evaporation rate, and the concentration of polymer and possible plasticizer further affect the

Correspondence to: H. Juvonen (helka.juvonen@abo.fi).
Contract grant sponsors: FinNano Program of TEKES, The Finnish Funding Agency for Technology and Innovation.

film formation and film properties.[8,30,31] Surface properties of films, for example, surface topography and phase separation, may also influence the barrier properties.[32,33] These surface properties can be studied by surface-sensitive methods. Atomic force microscopy (AFM) is a versatile tool to study surfaces and provide information of surface roughness, morphology, and phase contrasts in several types of biopolymer-based films.[34–38]

Transglutaminase (TGase) has widely been used for enzymatic crosslinking of several food protein or polysaccharide-containing films.[23,39–41] TGase is able to create isopeptide crosslinks between lysine and glutamine residues in protein chains or lysine groups in proteins and primary amino groups in nonproteins. Treatment with microbial TGase has been reported to improve mechanical and surface hydrophobic properties of protein films.[42] Oxidoreductases such as laccase and tyrosinase have also been shown to be able to crosslink milk, meat, and cereal proteins.[43–47] The high redox potential laccase from *Trametes hirsuta* (ThL) can also efficiently crosslink proteins in the presence of low-molecular-weight phenolic components as bridging agents.[48]

In this study, casein from bovine milk in the form of sodium caseinate was used to produce films and coatings for packaging applications. Sodium caseinate was crosslinked by oxidoreductases *Trametes hirsuta* laccase (ThL), *Trichoderma reesei* tyrosinase (TrTyr), or TGase. Biochemical characterization of enzymatic treatment of sodium caseinate was combined with surface studies of the protein films to analyze the different parameters that affect the properties of the coating.

EXPERIMENTAL

Materials

Sodium caseinate (protein content 96%) from KaslinkFoods was used as the substrate protein. Enzymes used for crosslinking of sodium caseinate were *Trametes hirsuta* laccase (ThL) and *Trichoderma reesei* tyrosinase (TrTyr). ThL was partially purified from concentrated culture supernatant using a DEAE anion exchange chromatography.[49] TrTyr was purified as described by Selinheimo et al.[50] Transglutaminase (Trademark CONNECT IT-E) was obtained from Ajinomoto (Japan), and further purification was carried out as described by Lantto et al.[45] Glycerol used as a plasticizer was obtained from Merck.

Methods and techniques

Preparation of caseinate films

The procedure to prepare protein-based films and coatings was modified from Hong and Krochta.[5] Sodium caseinate was dispersed in water (10%) by mixing with magnetic stirrer at room temperature for 10 min. Few drops of sodium hydroxide (5*M* NaOH) were added to adjust the pH to 7. The solution was heated to 90°C and further mixed for 30 min to denature the protein, and the plasticizer (glycerol) was added (33.3% based on the dry weight of the protein unless otherwise stated). The volume of the solution was adjusted to gain the final concentration of the protein (10% based on the dry weight), and the air bubbles were removed in an ultrasonic bath. Caseinate was crosslinked by the addition of ThL, TrTyr, or TGase to the solution. An enzyme dose of 500 or 1000 nkat/g protein was used. In some cases, the enzyme was applied by spraying the enzyme solution (concentration adjusted to correspond 0–20 nkat/cm^2) to the moist protein coating using a Nalgene spray bottle. Reference films without crosslinking agent were also prepared. Stand-alone films were prepared by casting the protein solution into Petri dishes or Teflon moulds (0.1 mL/cm^2 of 10% sodium caseinate was used). After casting, the drying of the film was prevented for predefined time (0–48 h at 23°C), which is regarded as the reaction time in the results section. Drying was prevented by enclosing the film into a moisture-impermeable pouch containing an open water vial to saturate the gas space of the pouch with water vapor. After this, the films were dried typically overnight (50% relative humidity (RH), 23°C) and stored in the same conditions enclosed in a polyethylene pouch.

Corona-treated three-layer PE film (ZCLL 100, Amcor Flexibles Finland Oy, 100 μm) and Cupforma Classic cardboard (Stora Enso) were coated with the casein films to demonstrate the use of protein coatings as packaging material. The coatings were applied on the packaging materials using a K Hand Coater (RK Print Coat Instruments). The thickness of wet protein layer was 100 μm. The coated cardboards were dried at 80°C and stored in 50% RH (23°C) enclosed in a polyethylene pouch.

Characterization of caseinate films

Activity assays and oxygen consumption. The activity of ThL was measured using ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) as the substrate at pH 4.5.[51] Activity of TrTyr was measured according to Robb,[52] using 15 m*M* 3,4-dihydroxy-L-phenylalanine as substrate. Enzymatic activity of both ThL and TrTyr toward sodium caseinate was confirmed by measuring the oxygen consumption of 1% sodium caseinate solution (pH 7) in the presence of ThL (100–10,000 nkat/g protein) or TrTyr (100–1000 nkat/g protein). Dissolved O_2 was monitored based on dynamic luminescence quenching using a Fibox 3

minisensor oxygen meter (PreSens, Germany) calibrated using 1% Na_2SO_3 (0%) and air-saturated H_2O (100%).

Gel electrophoresis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used for qualitative analyses of modified proteins. In the experiments, ready-made 12% polyacrylamide (w/v) gels (BioRad, Hercules, CA) were used. Prestained molecular weight standards 6.5–66 kDa and 7.3–211.8 kDa (BioRad, Hercules, CA) as well as 14.4–94 kDa, low-molecular-weight markers (Amersham Biosciences, Uppsala, Sweden) were used as molecular weight markers. Electrophoresis was carried out at room temperature using a constant current of 80 mA (U = 200 V). For protein detection, Blue Stain Reagent (Pierce Biotechnology, IL) was used.

Solubility tests. Solubility tests of the prepared films were carried out in water and succinate buffer. The films were soaked for 15 min, and the solubilization of the film was monitored by visual inspection. The weight loss of the visually insoluble films was also analyzed gravimetrically by measuring the weight loss of the film after 10 min. The amount of dissolved protein was determined using Bio-Rad Protein Assay (Bio-Rad laboratories, CA).

Contact angle measurements. Water contact angle measurements were performed by using CAM 200 device in room temperature unless otherwise mentioned. Contact angles were measured 2 s after droplet application.

AFM analyses. The phase contrast and surface topography analyses were performed with Nanoscope IIIa AFM and NTEGRA AFM instruments using the intermittent contact mode. The AFM probes Mikromasch NSC15/no Al were used. Images of 10 × 10 μm^2 size were captured. The Scanning Probe Image Processor (Image Metrology, Denmark) software was used for roughness analysis of the images. Out of a wide range of different roughness parameters, RMS (root mean square) roughness, that is, standard deviation of height values (Sq), was used in this work to describe the studied surfaces.

RESULTS AND DISCUSSION

Crosslink formation

The reactivity of oxidoreductases ThL and TrTyr toward a bulk sample of sodium caseinate was studied by measuring the consumption of the cosubstrate, that is, oxygen at pH 7 (Fig. 1). The crosslinking mechanism of TGase belonging to the group of transferases is different as described in the introduction and does not involve consumption of oxygen. When using the same enzyme dosage based on activity units, the oxygen consumption rate and hence the reaction rate was higher for TrTyr. The enzyme dosage for SDS-PAGE samples was based on oxygen consumption test of TrTyr. ThL and TGase were used in analogous concentrations. From an SDS-PAGE gel, it could be verified that ThL-treated films (1 nkat/mg protein) were crosslinked, and high-molecular-weight bands as well as weakening of the original protein bands were observed [Fig. 2(A)]. Some protein degradation was also observed as the amount of small protein fragments increased compared with reference samples probably because of oxidative degradation as discovered by Selinheimo et al.[44] for the oxidation of α-casein. In addition to the oxidative reduction caused by ThL, the protease contaminant in the enzyme may partly cause the digestion of the protein chains into shorter fragments.

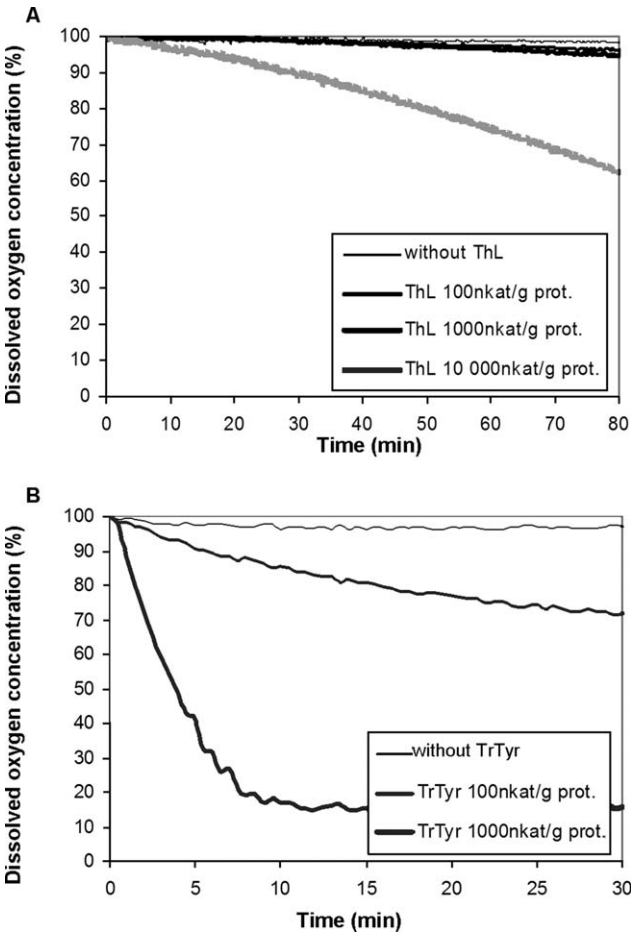

Figure 1 Oxidation of sodium caseinate catalyzed by ThL (A) and TrTyr (B) measured as the consumption of oxygen catalyzed. The sodium caseinate concentration in the experiments was 1%, and the pH of the reaction mixture was 7.

In the case of TrTyr treatment [Fig. 2(B)], high-molecular-weight reaction products were formed, and no degradation was observed. When 1 nkat/mg protein of TrTyr was used for the treatment, fully insoluble reaction product was obtained even if the film was allowed to dry immediately after casting (reaction time 0 h).

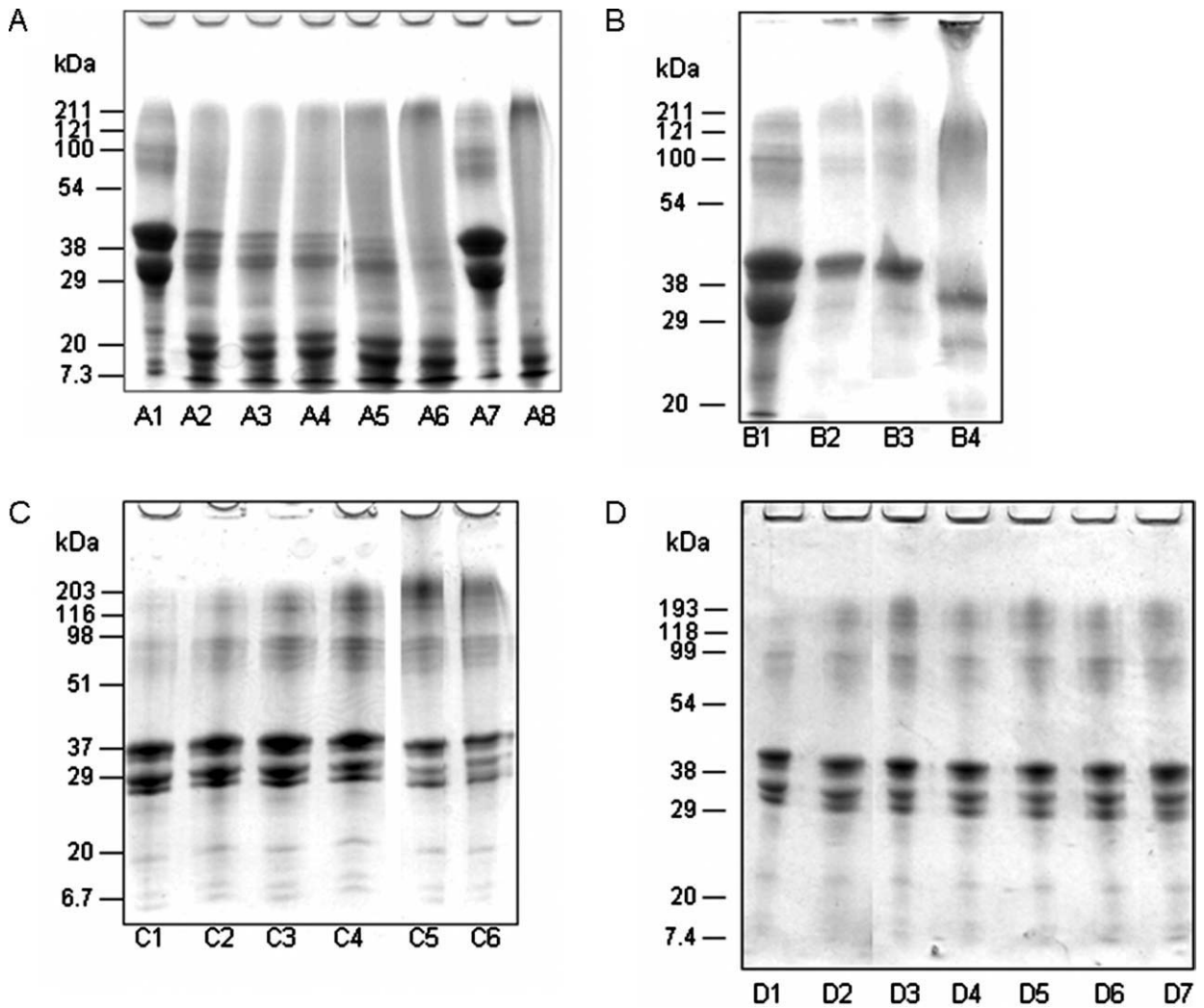

Figure 2 SDS-PAGE gel showing crosslinking of caseinate by ThL (1 nkat/mg) (A), TrTyr (0.1 mg/mg) (B), TGase (C), and TGase + TrTyr (D). Lanes: sodium caseinate without enzymatic treatment: 0 h (A1), 48 h (A7); caseinate + ThL: 0 h (A2), 2 h (A3), 6 h (A4), 12 h (A5), 24 h (A6), 48 h (A8); sodium caseinate without enzymatic treatment (B1); caseinate + TrTyr: 0 h (B2), 2 h (B3), 24 h (B4); sodium caseinate without enzymatic treatment (C1); caseinate + TGase: 0.005 nkat/mg (C2), 0.01 nkat/mg (C3), 0.025 nkat/mg (C4), 0.05 nkat/mg (C5), 0.5 nkat/mg (C6); sodium caseinate without enzymatic treatment (D1), caseinate + TrTyr (1 nkat/mg) (D2), caseinate + TrTyr (1 nkat/mg) + TGase (1 nkat/mg) (D3), caseinate + TrTyr (1 nkat/mg) + TGase (0.1 nkat/mg) (D4), caseinate + TrTyr (1 nkat/mg) + TGase (0.5 nkat/mg) (D5), caseinate + TGase (1 nkat/mg) (D6), caseinate + TrTyr (0.5 nkat/mg) + TGase (0.5 nkat/mg) (D7). Time indicated in the A and B lane means a time when drying of the film was prevented. Location of molecular weight markers is shown aside.

TGase was also studied as a reference enzyme, and the crosslinking pattern of the sodium caseinate with different enzyme doses (film allowed to dry immediately after casting, reaction time 0 h) is shown in Figure 2(C). Even if the crosslinking mechanisms of TrTyr and TGase are different, the crosslinking pattern of TGase seemed to be similar to that of TrTyr as verified in Figure 2(D).

Film formation, visual characterization, and solubility

Stand-alone films and coatings could be formed from all enzyme-treated sodium caseinate solutions; however, the properties of the films varied a lot depending on the treatment. ThL crosslinked films were soluble in water but did not dissolve in succinate buffer (pH 4.5). A shift in the pH dependency of the protein solubility might be an indication for a change in the isoelectric point of sodium caseinate after modification by ThL, because the solubility of sodium caseinate strongly depends on its isoelectric point.[53]

Interestingly, films made by adding TrTyr directly to the protein solution (1 nkat/mg protein or 5 nkat/mg protein), whereafter the films were casted according to the same procedure as the ThL films did not dissolve in water or succinate buffer. The solubility was also evaluated for films treated with

TABLE I
Effect of TrTyr Dose on the Solubility of Hand-Coated Casein Films Crosslinked by Spraying

Coating thickness (mg/cm^2)	TrTyr dose (nkat/mg)	Solubilized protein (%)
1.3	0.6	60
1.3	2.8	11
0.9	2.8	0

The amount of dissolved protein was measured after 15-min immersion of 4.9-cm^2 film per 3 mL H_2O. Reaction time before drying was 5 min, and the films were dried for 14–20 min at +80°C.

1 nkat/mg protein and dried immediately after TrTyr addition at 80°C. These films remained visually insoluble indicating a very rapid reaction catalyzed by TrTyr because the drying at high temperature is likely to inactivate the TrTyr rapidly. It was also observed that already low dosage of 0.1 nkat TrTyr/mg protein clearly delayed the dissolving of protein film. Gravimetric tests revealed the dissolution of most of the plasticizer as expected (20–22% weight loss because of the 10-min immersion of the film in H_2O).

The effect of TrTyr-catalyzed crosslinking on coating properties was also studied by spraying TrTyr on top of a moist protein coating on cardboard. The crosslinking was allowed to proceed for 5 min before drying the coating rapidly at 80°C. No protein solubilization could be observed after treating a 0.9 mg/m^3 coating with TrTyr dose of 2.8 nkat/mg protein. This demonstrated that insoluble coatings could also be formed using this method even if the drying is likely to inactivate TrTyr rapidly (Table I).

Insoluble stand-alone films were also formed with TGase treatment. An enzyme dose of 0.5 nkat/mg of casein was required to obtain a visually insoluble film. Analogously to caseinate crosslinking by TrTyr,

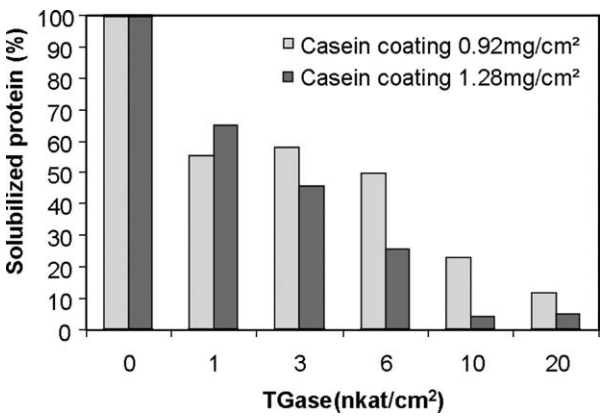

Figure 3 Effect of TGase dose on the solubility of hand-coated casein films crosslinked by spraying. The reaction time before drying was 2 min, and the drying time was 14–20 min at +80°C. The solubilized protein (%) was measured after 15-min immersion of the film in water.

TABLE II
Effect of TrTyr Treatment on the Color of the Casein-Based Stand-Alone Films (Average of Five Measurements)

	Colorimetric values		
TrTyr dose (nkat/mg)	*L* (black-white)	*a* (red-green)	*b* (yellow-blue)
0	90	−4	7
0.1	81	−7	27
1.0	66	−4	42

Glycerol was used as a plasticizer. Colorimetric values were measured with Minolta Chroma Meter using (*L*, *a*, *b*) scale.

crosslinking of the caseinate coatings by TGase was also studied by spraying the enzyme on the coating before drying. Interestingly, fully insoluble films could not be obtained with TGase treatment in this way, but a considerable decrease in the coating solubility could be obtained already with the dose of ~ 1 nkat/mg protein (Fig. 3).

ThL and TGase formed colorless, opaque films, whereas TrTyr films were colored. The intensity of the color of TrTyr films depended on the amount of added enzyme being lighter (yellow) with smaller TrTyr doses and brownish when more enzyme was used (Table II). Color formation during tyrosinase-catalyzed protein crosslinking has been reported and discussed also previously.[54]

Contact angles

The water contact angles of the enzymatically crosslinked protein films were measured. When the reaction time (i.e., film drying time) was 24 h, the contact angle of ThL-crosslinked Na-caseinate films decreased, that is, the surface became more hydrophilic (Table III). Partial degradation of protein induced by ThL and protease contaminant may have increased the wettability of the crosslinked films, laccase-treated films. However, this decrease in water contact angle with increased reaction time was also observed for the reference sample without crosslinking, indicating that the change in the wetting

TABLE III
Effect of ThL-Catalyzed Crosslinking on the Contact Angle of the Caseinate-Based Stand-Alone Films

Reaction time (h)	Contact angle of reference film (°)	Contact angle of ThL-treated film (°)
0	81	84
2	86	89
6	90	80
24	71	68

ThL dose of 1 nkat/mg of casein was used. Contact angles were measured at 23°C and 50% relative humidity. Glycerol was used as a plasticizer. ($n = 3$, STD 0.7–4.3).

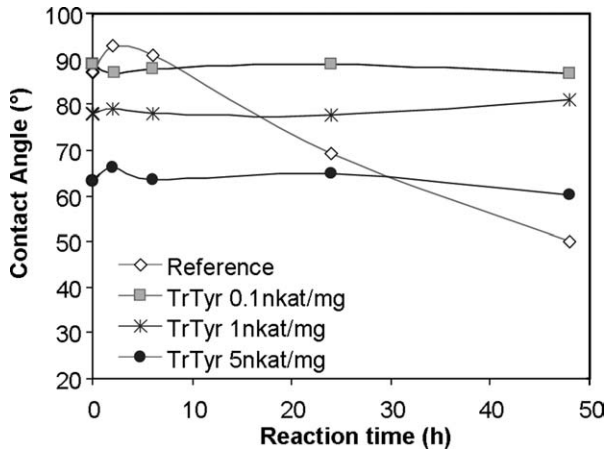

Figure 4 Contact angles of TrTyr crosslinked casein films as a function of the reaction time. The reaction time is a predefined time when drying of the film was prevented. Glycerol (33.3%) was used as plasticizer. (n = 3–7, STD = 1.3–15.9 for reference samples, STD = 1.4–6.2 for TrTyr-treated samples).

properties was resulting from the phase separation of the small molecules in sodium caseinate. Another possible reason is the reorientation of the hydrophilic side chains of the protein toward the surface during the film formation and drying.

Contact angles of the TrTyr-crosslinked Na-caseinate stand-alone films decreased with increasing TrTyr concentration as can be seen in Figure 4. Furthermore, the TrTyr treatment stabilizes the contact angle of the film with increasing reaction time, so that the water contact angle remains at the same level independent of the reaction time. The increased hydrophilicity with an increasing TrTyr dosage is due to its oxidation ability of phenol groups in casein. A higher TrTyr content leads to a higher extent of hydrophilic groups on the surface of the film. The independence of the CA on the reaction time results from the fast oxidation of caseinate by TrTyr and the subsequent crosslinking step. The wetting behavior of the TrTyr-treated films with the increasing drying time differs from the wetting of the films without enzymatic treatment and the wetting of the films treated with ThL.

Atomic force microscopy

The development of different phases in the films was studied using AFM. The phase contrast was homogeneous for both reference and ThL-treated; glycerol-containing films dried immediately at 23°C after film casting (i.e., when the reaction time was 0 h) [Fig. 5(A)]. However, if the reaction was forced further by delaying the drying, a clear contrast in the phase images of ThL-treated films was observed, indicating phase separation (reaction time 6 or 24 h). The corresponding reference samples without enzyme treatment appeared clearly more homogeneous, but also these films became more heterogeneous with increasing reaction time.

A corresponding study with films without any plasticizer showed no contrast in the phase images for the nonenzyme-treated films immediately after film casting [Fig. 5(B)]. Signs of phase separation were observed for films with reaction times 6 or 24 h, indicating that the phase separation at longer reaction times is not only due to the plasticizer, the ThL treatment, or protease contaminant but potentially also partly due to the phase separation of the smaller molecular fragments in sodium caseinate (Fig. 2).

As described above, the TrTyr treatment resulted in an increased hydrophilicity of the films with an increasing amount of TrTyr and subsequent stabilization of the wetting of the film (Fig. 4). This was supported by the AFM phase contrast studies shown in Figure 6. Treatment of the films with TrTyr (0.1 nkat/mg protein and 1 nkat/mg protein) resulted in homogeneous phase images independent of the reaction time, whereas the phase images for the reference samples appeared heterogeneous (reaction time 6 or 24 h).

Our AFM results supported the results obtained by gel electrophoresis (SDS-PAGE) and contact angle analysis. The fact that phase separation occurred also in the reference films without enzymatic treatment (although as a weaker phenomenon) is likely to be due to the lack of crosslinking of the film, when the smaller molecules present in sodium caseinate may move easier and form separate phases. ThL treatment results in partial degradation of the protein, which is likely to have an effect on the increasing hydrophilicity of the film as well as on the pronounced phase separation taking place on the film surface. ThL-treated caseinate films and caseinate films without enzymatic treatment, both with and without glycerol as plasticizer, showed phase separation as a result of the increased reaction time. On the other hand, the contact angle measurements as well as the AFM analysis revealed no phase separation for TrTyr crosslinked films, showing that sufficient crosslinking and nonexisting protein degradation resulted in a more stable film. According to the AFM phase and topography images of caseinate films treated with either TGase or TrTyr (data not shown), both enzymes resulted in films of similar roughness (S_q) with no phase separation in TGase-treated samples analogously to TrTyr-treated samples.

CONCLUSIONS

ThL, TrTyr, and TGase were tested for their ability to crosslink sodium caseinate. All three enzymes

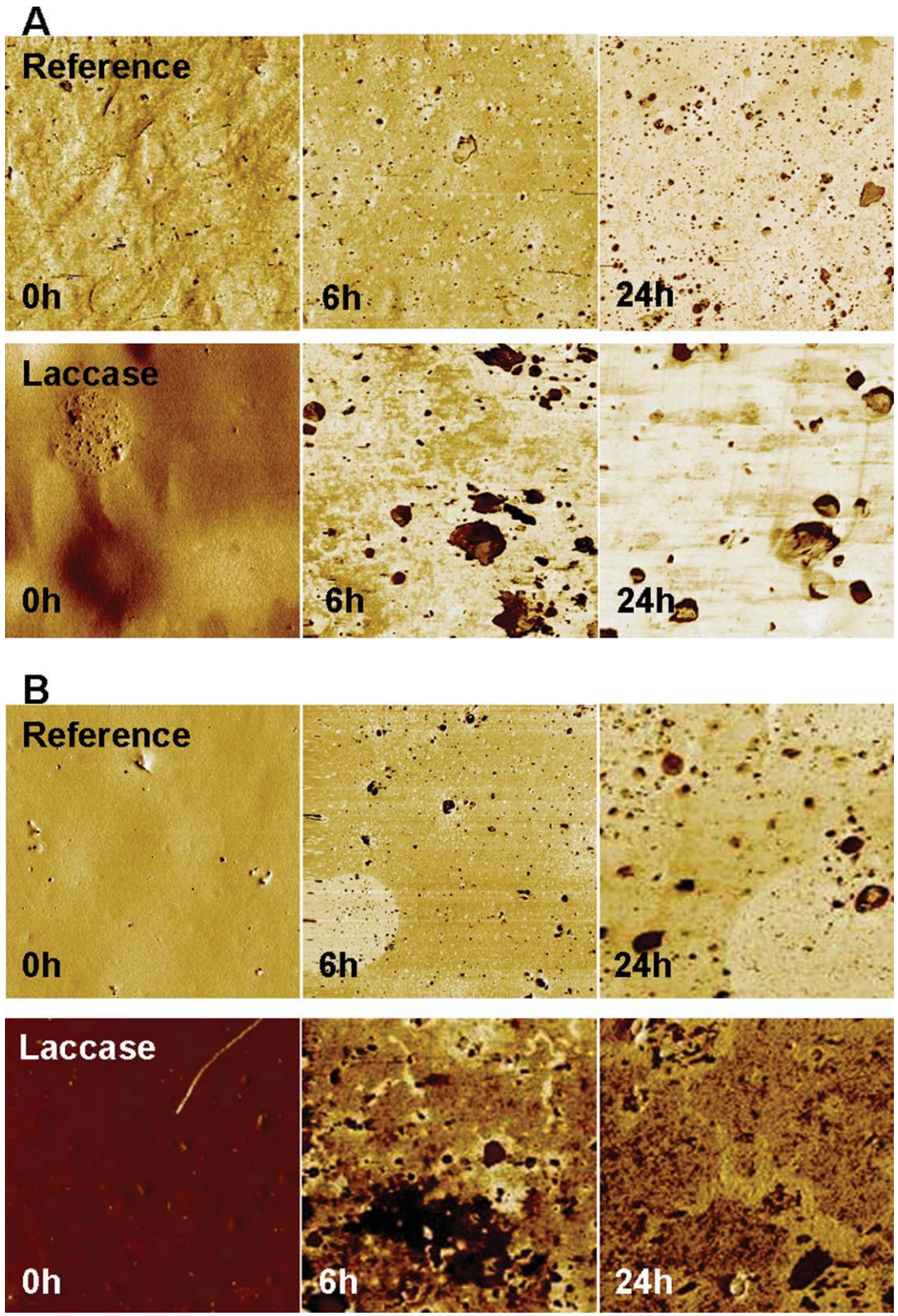

Figure 5 AFM phase contrast images of caseinate films containing glycerol as plasticizer (A) and caseinate films without plasticizer (B). Caseinate films are made either without enzymatic treatment (reference) or with ThL treatment (laccase). Phase images (image size 10 μm × 10 μm) of samples with reaction times of 0, 6, and 24 h are shown. Reaction time is defined as the predefined time when drying of the film was prevented. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

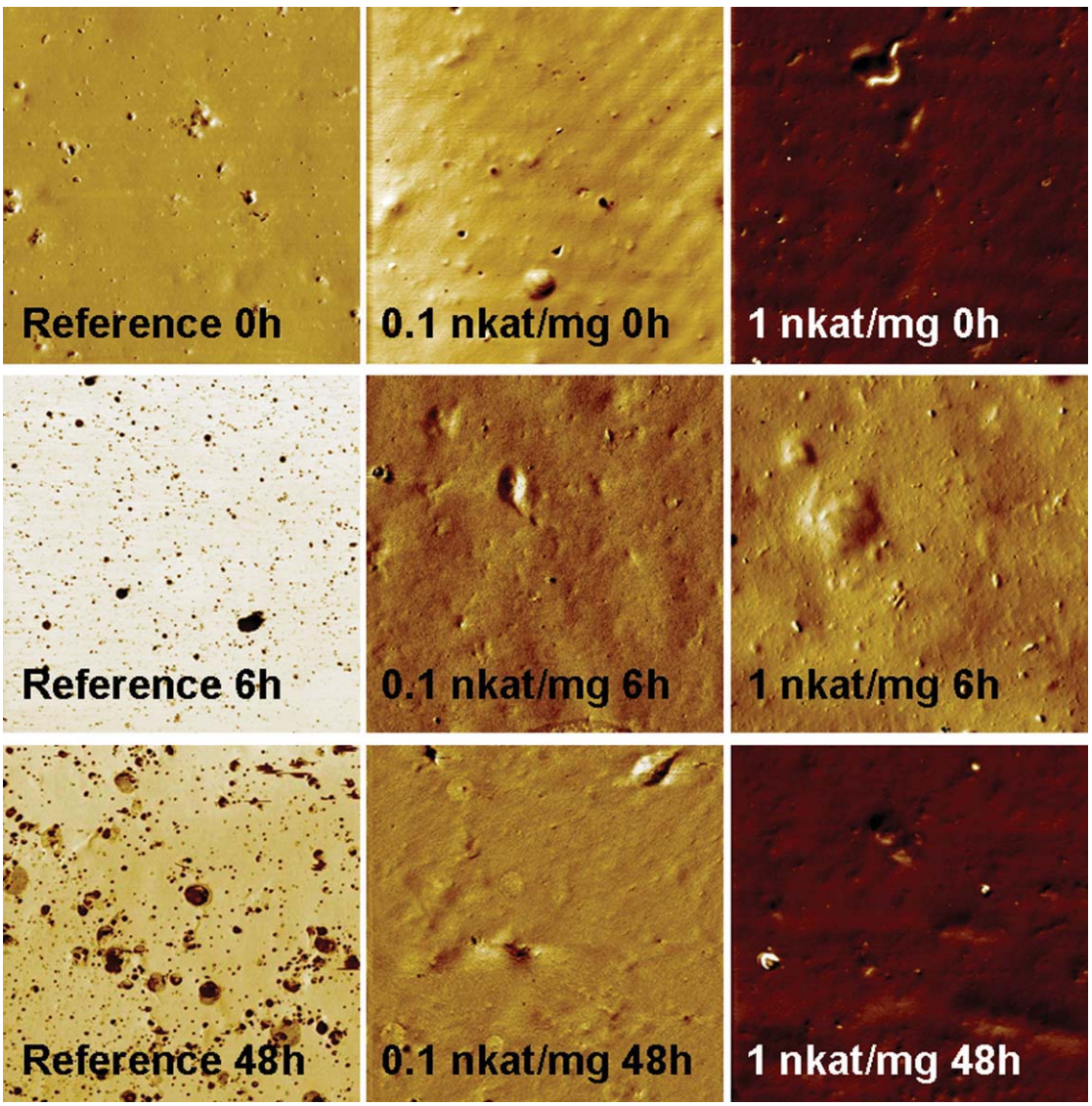

Figure 6 AFM phase contrast images of caseinate films without enzymatic treatment (reference), with 0.1 nkat/mg TrTyr and 1 nkat/mg TrTyr. Phase images (image size 10 μm × 10 μm) of samples with reaction times of 0, 6, and 48 h are shown. Glycerol (33.3%) was used as a plasticizer. Reaction time is defined as a predefined time when drying of the film was prevented. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

were active toward sodium caseinate and were able to form chemically different intermolecular bonds between the caseinate molecules. Stand-alone films and coatings were successfully formed from solutions containing enzymatically treated sodium caseinate. Films treated with ThL were still soluble in water, but TrTyr or TGase converted the protein films into insoluble ones. No protein degradation or phase separation occurred in these insoluble films with increasing reaction time. This shows that TrTyr similarly to TGase is an effective new crosslinking enzyme, which can form insoluble films with a different type of crosslinking chemistry. Combination of different chemistries gives new possibilities to create even further modified films in the future and thereby modulate the film properties. Crosslinking of a sodium caseinate-based paperboard coating by TrTyr applied by spraying was also successfully demonstrated. The possibility to spray the crosslinking agent and subsequently dry it rapidly clearly indicated the potential to apply the enzymatic crosslinking of protein-based coating as a reel-to-reel process. As a summary, our experiments resulted in an interesting new insoluble type of biopolymer-based coating with a packaging application potential.

Mariitta Svanberg (VTT) is thanked for excellent technical assistance. Kristiina Kruus, Martina Andberg, and Birgit Hillebrandt-Chellaoui (VTT) are thanked for supplying the ThL and TrTyr enzyme preparations. Raija Lantto and Nina Vihersola (VTT) are thanked for supplying the TGase enzyme preparation.

References

1. Tharanathan, R. N. Trends Food Sci Technol 2003, 14, 71.
2. Weber, C. J., Ed. Biobased Packaging Materials for the Food Industry—Status and Perspectives. A European Concerted Action, KVL, Denmark 2000; Vol. 1.
3. Siracusa, V.; Rocculi, P.; Romani, S.; Dalla Rosa, M. Trends Food Sci Technol 2008, 19, 634.
4. Anker, M.; Stading, M.; Hermansson, A. M. J Agric Food Chem 2000, 48, 3806.
5. Hong, S. I.; Krochta, J. M. J Food Eng 2006, 77, 739.
6. Rhim, J.-W.; Perry, K. W. Crit Rev Food Sci Nutr 2007, 47, 411.
7. Khwaldia, K.; Perez, C.; Banon, S.; Desobry, S.; Hardy, J. Crit Rev Food Sci Nutr 2004, 44, 239.
8. Miller, K. S.; Krochta, J. M. Trends Food Sci Technol 1997, 8, 228.
9. Paramawati, R.; Yoshino, T.; Isobe, S. Food Sci Technol Res 2001, 7, 191.
10. Irissin-Mangata, J.; Boutevin, B.; Bauduin, G. Polym Bull 1999, 43, 441.
11. Cho, S. Y.; Park, J.-W.; Batt, H. P.; Thomas, R. L. LWT—Food Sci Technol 2007, 40, 418.
12. Farris, S.; Introzzi, L.; Piergiovanni, L. Packag Technol Sci 2009, 22, 69.
13. Gómez-Guillén, M. C.; Pérez-Mateos, M.; Gómez-Estaca, J.; López-Caballero, E.; Giménez, B.; Montero, P. Trends Food Sci Technol 2009, 20, 3.
14. Krochta, J. M. In Food Proteins and Their Applications; Damodaran, S., Paraf, A., Eds.; Marcel Dekker: New York, 1997; p 529.
15. Tomasula, P. M.; Craig, J. C., Jr.; Boswell, R. T.; Cook, R. D.; Kurantz, M. J.; Maxwell, M. J Dairy Sci 1995, 78, 506.
16. Hofland, G. W.; Berkhoff, M.; Witkamp, G. J.; Van der Wielen, L. A. M. Int Dairy J 2003, 13, 685.
17. Kozempel, M.; Tomasula, P. M. J Agric Food Chem 2004, 52, 1190.
18. Jagannath, J. H.; Nanjappa, C.; Das Gupta, D. K.; Bawa, A. S. J Appl Polym Sci 2003, 88, 64.
19. Cozmuta, I.; Blanco, M.; Goddard, W. A. J Phys Chem B 2007, 111, 3151.
20. Arvanitoyannis, I.; Kalichevsky, M.; Blanshard, J. M. V. Carbohydr Polym 1994, 24, 1.
21. Subramanian, S.; Sampath, S. Biomacromolecules 2007, 8, 2120.
22. Klopffer, M. H.; Flaconnèche, B. Oil Gas Sci Technol 2001, 56, 223.
23. Di Pierro, P.; Chico, B.; Villalonga, R.; Mariniello, L.; Damiao, A. E.; Masi, P.; Porta, R. Biomacromolecules 2006, 7, 744.
24. Di Pierro, P.; Chico, B.; Villalonga, R.; Mariniello, L.; Masi, P.; Porta, R. Enzyme Microb Technol 2007, 40, 437.
25. Chambi, H.; Grosso, C. Food Res Int 2006, 39, 458.
26. Ouattara, B.; Canh, L. T.; Vachon, C.; Mateescu, M. A.; Lacroix, M. Radiat Phys Chem 2002, 63, 821.
27. Rhim, J. W.; Gennadios, A.; Fu, D.; Weller, C. L.; Hanna, M. A. Lebensm-Wiss Technol 1999, 32, 129.
28. Hernández-Muñoz, P.; Villalobos, R.; Chiralt, A. Food Hydrocolloids 2004, 18, 403.
29. Orliac, O.; Rouilly, A.; Silvestre, F.; Rigal, L. Polymer 2002, 43, 5417.
30. Lefévre, T.; Subirade, M.; Pézolet, M. Biomacromolecules 2005, 6, 3209.
31. Arvanitoyannis, I. S.; Nakayama, A.; Aiba, S. Carbohydr Polym 1998, 37, 371.
32. Ghanbarzadeh, B.; Oromiehi, A. R. Int J Biol Macromol 2008, 43, 209.
33. Petersson, M.; Loren, N.; Stading, M. Biomacromolecules 2005, 6, 932.
34. Mathew, S.; Abraham, T. E. Food Hydrocolloids 2008, 22, 826.
35. Rindlav-Westling, A.; Gatenholm, P. Biomacromolecules 2003, 4, 166.
36. Zuo, Y. Y.; Keating, E.; Zhao, L.; Tadayyon, S. M.; Veldhuizen, R. A. W.; Petersen, N. O.; Possmayer, F. Biophys J 2008, 94, 3549.
37. Bantchev, G. B.; Schwartz, D. K. Langmuir 2004, 20, 11692.
38. Müller-Buschbaum, P.; Gebhardt, R.; Maurer, E.; Bauer, E.; Gehrke, R.; Doster, W. Biomacromolecules 2006, 7, 1773.
39. Kuraishi, C.; Yamazaki, K.; Susa, Y. Food Rev Int 2001, 17, 221.
40. Gauche, C.; Vieira, J. T. C.; Ogliari, P. J.; Bordignon-Luiz, M. T. Process Biochem 2008, 43, 788.
41. Boenisch, M. P.; Huss, M.; Weitl, K.; Kulozik, U. Int Dairy J 2007, 17, 1360.
42. Tang, C.-H.; Jiang, Y. Food Res Int 2007, 40, 504.
43. Steffensen, C. L.; Andersen, M. L.; Degn, P. E.; Nielsen, J. H. J Agric Food Chem 2008, 56, 12002.
44. Selinheimo, E.; Lampila, P.; Mattinen, M.-L.; Buchert, J. J Agric Food Chem 2008, 56, 3118.
45. Lantto, R.; Puolanne, E.; Kalkkinen, N.; Buchert, J.; Autio, K. J Agric Food Chem 2005, 53, 9231.
46. Thalmann, C. R.; Lötzbeyer, T. Eur Food Res Technol 2002, 214, 276.
47. Mattinen, M.-L.; Lantto, R.; Selinheimo, E.; Kruus, K.; Buchert, J. J Biotechnol 2008, 133, 395.
48. Selinheimo, E. Tyrosinase and laccase as novel crosslinking tools for food biopolymers, Dissertation for the degree of Doctor of Science in Technology; VTT Publications: Espoo, 2008; p 693.
49. Frasconi, M.; Favero, G.; Boer, H.; Koivula, A.; Mazzei, F. Proteins Proteomics 2010, 1804, 899.
50. Selinheimo, E.; Saloheimo, M.; Ahola, E.; Westerholm-Parvinen, A.; Kalkkinen, N.; Buchert, J.; Kruus, K. FEBS J 2006, 273, 4322.
51. Niku-Paavola, M. L.; Karhunen, E.; Salola, P.; Raunio, V. Biochem J 1988, 254, 877.
52. Robb, D. A. In Copper Proteins and Copper Enzymes; Lontie, R., Ed.; CRC Press: Boca Raton, 1984; p 207.
53. Ma, H.; Forssell, P.; Partanen, R.; Seppänen, R.; Buchert, J.; Boer, H. J Agric Food Chem 57, 2009, 3800.
54. Monogioudi, E.; Creusot, N.; Kruus, K.; Gruppen, H.; Buchert, J.; Mattinen, M. L. Food Hydrocolloids 2009, 23, 2008.