

Mechanical Properties, Water Vapor Permeability, and Moisture Contents of β -Lactoglobulin and Whey Protein Films Using Multivariate Analysis

Martin Anker, Mats Stading,* and Anne-Marie Hermansson

SIK—The Swedish Institute for Food and Biotechnology, P.O. Box 5401, S-402 29 Göteborg, Sweden

Mechanical and barrier properties of β -lactoglobulin (β -Lg) and whey protein isolate (WPI) films were studied using sorbitol (S) as a plasticizer. The films were cast from heated aqueous solutions and dried in a climate chamber at 23 °C and 50% relative humidity for 16 h. The multivariate analysis used has proved to be a valuable tool for evaluating and quantifying the influences of the variables in the specified experimental domain. Two identical factorial designs were applied to evaluate the influence of the concentration of β -Lg and WPI, the concentration of S, and the pH. The two materials, β -Lg and WPI, show similar results, which can be attributed to the dominating protein β -lactoglobulin. At pH 9, Young's modulus and stress at break are not affected when the concentration of β -Lg, WPI, or S varies. At pH 7 and 8, Young's modulus and stress at break increase when the concentration of β -Lg and WPI increases, and they decrease when the concentration of S increases. Strain at break increases when pH increases from 7 to 9, a more pronounced effect being observed for the WPI films. Water vapor permeability (WVP) decreases and increases for pH 7 and 9, respectively, as the concentration of β -Lg and WPI increases. This contrast in behavior at different pH values is probably due to a structural difference that occurs above pH 8. Moisture content and WVP increase when S increases. Here a clear distinction can be observed between the two film materials: the β -Lg films show higher values for both moisture content and WVP measurements.

Keywords: β -Lactoglobulin; whey protein; mechanical properties; water vapor permeability; moisture content; multivariate analysis

INTRODUCTION

Whey proteins are a byproduct of cheese-making and have generally been disposed of as animal feed or used in infant formulas, sports food, clinical nutrition products, etc. Today, great efforts are being made to find new uses for whey protein, for example, as edible and biodegradable films. The interest in the study of edible and biodegradable films has increased during the past decade, one reason being that edible and biodegradable films are environmentally friendly alternatives to synthetic, nonbiodegradable packaging films. Although it is not feasible to entirely replace synthetic packaging films, edible and biodegradable films do have the potential to reduce and replace synthetic packaging films in some applications. Current research on the properties of edible and biodegradable films and potential applications has been outlined in recent reviews (Krochta et al., 1994; Anker, 1996; Gennadios et al., 1997; Krochta and De Moulder-Johnston, 1997; Miller and Krochta, 1997).

The present study on β -lactoglobulin (β -Lg) and whey protein films was promoted by the work previously performed on β -lactoglobulin and whey protein gels in our laboratory. The findings from these studies have been used as a basis to produce films and to obtain further data that can be used in future food-packaging applications. β -Lactoglobulin is the dominating protein of the whey proteins and tends to influence the behavior of whey protein gels. The microstructure and physical properties of whey protein gels are sensitive to the heating conditions, amount of salt addition, pH, and

concentration of whey protein, as well as the concentration of other additives such as plasticizers. The heating rate has therefore been held constant in this investigation, no salt has been added, and the influence of the other factors will be elucidated in this paper (Hermansson, 1979, 1986; Langton and Hermansson, 1992; Stading and Hermansson, 1990, 1991; Stading et al., 1992, 1993).

Whey protein forms gels on heating and can form two types of gel network structures: fine-stranded and particulate (Langton and Hermansson, 1992; Stading and Hermansson, 1990). The isoelectric point of the whey proteins is around pH 5.2. Between approximately pH 4 and 6, the repulsion in the system is small, and white and opaque gels are formed, with network strand dimensions on the order of micrometers, that is, particulate gels. The particulate gel network is composed of almost spherical aggregates linked together and forming the threads of the network. At high and low pH values, the repulsion in the system is high, leading to the formation of more transparent gels with network strand dimensions of ca. nanometers, that is, fine-stranded gels. Langton and Hermansson (1992) found that the fine-stranded gels formed at low pH (pH <4) were composed of short stiff strands, whereas the fine-stranded structures at high pH (pH >6) had longer, more flexible strands. This is in agreement with results from Stading and Hermansson (1991), who found it possible to differentiate between particulate and fine-stranded gels using viscoelastic measurements. Large deformation measurements showed that fine-stranded gels at low pH had properties different from those of

the fine-stranded gels formed at high pH: the gels were brittle at low pH and rubber-like at high pH. Hermanson (1986) showed that the microstructure of whey protein gels at pH 9 was finer than that of the more aggregated structure at pH 7. Films produced at different pH values in a pilot study preceding the present investigation showed behavior that was similar to that of the gels presented above. All films were cast from heated aqueous solutions and dried at room conditions. At pH 4–6 the films were white and opaque, at pH <4 they were transparent and fragile, whereas at pH >6 the films were both transparent and flexible. Films formed at pH 10 were transparent and flexible, but the aqueous solutions tended to be a little yellowish. Hence, a pH range from 7 to 9 was chosen to study how pH affects the mechanical and barrier properties of practically usable films.

All β -lactoglobulin and whey protein films need to have some kind of plasticizer to induce sufficient flexibility and to avoid cracking of the films during the drying process. The drying process is an important parameter, and constant "normal" room conditions, for example, 23 °C and 50% relative humidity (RH), seem to reduce crack formation in films. The plasticizers often used in edible and biodegradable films are polyols such as glycerol, sorbitol, and propylene glycol. McHugh et al. (1994) concluded that sorbitol was more effective than glycerol as a plasticizer in that films of whey protein had lower oxygen permeabilities when plasticized with sorbitol at low and intermediate ($\leq 60\%$) RH. At high (>70%) RH the oxygen permeability curves of both glycerol and sorbitol were superimposed. Hence, sorbitol was used in this investigation as it binds less water than glycerol and imparts a better barrier against water vapor at low and intermediate RH. The concentrations of β -lactoglobulin, whey protein, and sorbitol were varied between 10 and 14% (w/w) based on the dry weight to give a multivariate design with reproducible films with enough durability and flexibility in each design point. Higher β -lactoglobulin or whey protein concentrations tended to gel during the heating process and made it difficult to cast films.

The aim of this paper was to compare the mechanical properties, water vapor permeability (WVP), and moisture content (MC), as well as the influence of the protein β -lactoglobulin, in films made of β -Lg and whey protein isolate (WPI). The influence of the three variables: concentration of β -Lg and WPI, concentration of S, and pH, were studied. Both quantitative and multivariate analyses were used to evaluate and interpret the results.

MATERIALS AND METHODS

Materials. β -Lg and WPI were obtained from MD Foods Ingredients (Videbaek, Denmark). β -Lg (PSDI-2400) is a native WPI with a very high content of β -lactoglobulin obtained by fractionation. The β -Lg powder had a dry content of $92 \pm 2\%$ protein ($N \times 6.38$), 1% fat, 0.2% lactose, and 5.5% moisture and a pH between 6.5 and 7.0 (0.10% solution). WPI (Lacprodan PSDI-9224) is a functional WPI used for protein fortification of clinical nutrition products, infant formulas, and sports foods. The WPI powder had a dry content of $93 \pm 2\%$ protein ($N \times 6.38$), 0.2% fat, 0.2% lactose, 5.5% moisture, and 4.0% minerals and a pH between 6.5 and 7.0 (0.10% solution). Sorbitol (S) (>98% purity) was used as a plasticizer and was obtained from Sigma Chemical Co. (St. Louis, MO). The concentration and properties of proteins in the β -Lg and WPI powders are listed in Table 1.

Table 1. Concentrations and Properties of Proteins in the β -Lg and WPI Powder

protein	β -Lg ^a (%)	WPI ^a (%)	molecular mass ^b (Da)	cysteine groups ^b
β -Lg ^c	>95	74	18600	2 (1-SH)
α -La ^d	<5	18	14200	4
BSA ^e	0	6	66000	17 (1-SH)
Ig ^f	0	2	150000–1000000	32

^a From MD Foods Ingredients (1997). ^b From Kinsella and Whitehead (1989). ^c β -Lactoglobulin. ^d α -Lactalbumin. ^e Bovine serum albumin. ^f Immunoglobulins.

Table 2. Reduced, Three Level, Factorial Design

run order	expt name	β -Lg and WPI % (w/w)	S % (w/w)	pH
1	N4a	14	14	9
2	N4b	14	14	9
3	N4c	14	14	9
4	N5a	12	12	8
5	N5b	12	12	8
6	N5c	12	12	8
7	N6a	12	12	8
8	N6b	12	12	8
9	N6c	12	12	8
10	N3a	10	14	7
11	N3b	10	14	7
12	N3c	10	14	7
13	N7a	12	12	8
14	N7b	12	12	8
15	N7c	12	12	8
16	N2a	10	10	9
17	N2b	10	10	9
18	N2c	10	10	9
19	N1a	14	10	7
20	N1b	14	10	7
21	N1c	14	10	7

Film Formation. The concentrations of β -Lg, WPI, and S were varied between 10 and 14% (w/w) according to Table 2. The solutions were mixed and dissolved with distilled deionized water at room temperature for 1 h. Solution pH was adjusted with 1 M sodium hydroxide after the mixing and before the heat treatment. The concentration of the solutions was compensated for the amount of sodium hydroxide added as well as the moisture content in the β -Lg, WPI, and S powder. A vacuum was applied at 10 min to remove dissolved air. The solutions were heated in an oil bath to 76.5, 70.5, and 68 °C for pH 7, 8, and 9, respectively. The temperature of the oil bath was changed accordingly to yield a constant total heating rate of 5.5–7.7 °C/min. This resulted in an average heating time of 9–13 min. The solutions gelled at different temperatures, depending on the concentration and pH, and therefore different maximum temperatures for each pH were used. Hence, the solutions were cast at 10 °C above the denaturation temperature of β -lactoglobulin. The denaturation temperatures were determined, by using differential scanning calorimetry, as 66.5, 60.5, and 58 °C for pH 7, 8, and 9, respectively (Hegg, 1980). Films were cast by pipetting solution onto a polystyrene Petri dish with an internal diameter of 14 cm. The Petri dish was covered with Bytac, which is a hydrophobic surface of Teflon FEP film laminated to an aluminum support layer (Norton Performance Plastics Corp., Akron, OH). The Teflon FEP film has a very low coefficient of friction and excellent release and cleanup characteristics. To minimize thickness variations, 15.4 g of solution was applied to each Petri dish. The cast solutions were allowed to cool and dry at room temperature for ≈ 4 h and were then dried in a climate chamber (LabRum Klimat Ab, Stockholm, Sweden) at 23 °C and 50% RH for 16 h. Dried films could be peeled intact from the casting surface. Films were preconditioned in the climate chamber at 23 °C and 50% RH for at least 48 h prior to all testing.

Film Thickness. Before testing, the thickness of the films was measured by a digital micrometer (model IDC-112CB, Mitutoya Corp., Tokyo, Japan) at five positions: one at the

center and four around the perimeter. The mechanical properties and WVP were calculated using the average thickness for each film replicate.

Mechanical Properties. A texture analyzer (model TA-XT2, Stable Micro Systems, Godalming, England) was used to determine mechanical properties in tension in accordance with ASTM D882-91. Initial grip separation and crosshead speed were set at 50 mm and 24 mm/min, respectively. The tested film strips were 80 mm long and 6 mm wide. Force and elongation were recorded during extension, and Young's modulus (E), stress at maximum force (σ_y), stress at break (σ_b), and strain at break (ϵ_b) were determined. The properties derived from the tensile test are often also referred to in the literature as modulus of elasticity, yield strength, tensile strength, and elongation. Yield strength is the tensile stress at which the first sign of nonelastic deformation occurs. However, the most frequently reported mechanical properties are σ_b and ϵ_b (Chen, 1995).

Moisture Content. MC was determined after drying in an oven (model T6060, Heraeus, Mölndal, Sweden) at 105 °C for 24 h. Glass Petri dishes were used as test containers. Small test specimens were cut and put on the Petri dishes, and the weights before and after oven drying were recorded. At least eight replicates of each design point were evaluated. MC was calculated as the percentage of weight loss based on the original weight, in accordance with ASTM D664-94.

Water Vapor Permeability. WVP was determined according to ASTM E96-90 and corrected for the stagnant air gap inside test cups for hydrophilic films using the WVP correction method (McHugh et al., 1993). A climate chamber containing a fan was used to test WVP, and the fan speed was set to achieve an air velocity of at least 152 m/min above the test cups to avoid stagnant air and ensure uniform RH inside the climate chamber. The climate chamber was set at 23 °C and 50% RH. The cups used to determine WVP were made in the workshop at SIK, by modifying a model described by Gennadios et al. (1994b). Circular cups were made from polytetrafluoroethylene (Teflon). Each consisted of a cylindrical bottom, a lid and a rubber O-ring. The bottom had a diameter of 10.0 cm. A well 7.3 cm in diameter and 2.0 cm in depth was milled into the bottom. An O-ring with an internal diameter of 7.8 cm was placed in a groove milled around the well. Cup walls were sufficiently thick to render the cup impermeable to water vapor. Four screws, symmetrically placed around the cup perimeter, were used to seal films between the lid and the bottom part, and good sealing of the films was provided by the O-ring. Distilled deionized water was placed in the bottom of the test cups to expose the film to a high RH inside the test cups. Using the WVP correction method, a fixed amount of water was used so that the stagnant air gap height inside the cups was <14 mm to ensure accurate determination of WVP. After the films were mounted, the whole assembly was weighed and placed in the climate chamber. Weighing (with an accuracy of 0.001 g) was repeated at 30 min intervals. Steady state was achieved in <2 h, and thereafter the weight of the assembly was recorded every 2 h and finally after 24 h. At least five replicates of each design point were evaluated. In all cases, a linear regression of the steady-state data yielded R^2 values >0.999. Due to the stagnant air gap inside the cups, the RH at the underside of the films was 72%; consequently, a 50–72% RH gradient was applied.

Multivariate Design and Statistics. A reduced, three-level, factorial design was used to evaluate each main effect as well as interaction effects (Figure 1). Since two materials, β -Lg and WPI, were examined in this study, two identical factorial designs were applied. Three variables were evaluated: the concentration of β -Lg and WPI (x_1), the concentration of sorbitol (x_2), and the pH (x_3). Four experimental points (N1–N4) and three central points (N5–N7) were measured for each design with three replicates of each measuring point. Table 2 presents the complete design for the two materials. Measured responses were E , σ_y , σ_b , and ϵ_b , as well as MC and WVP.

The data from the responses were analyzed using principal component analysis (PCA). PCA is a well-known technique

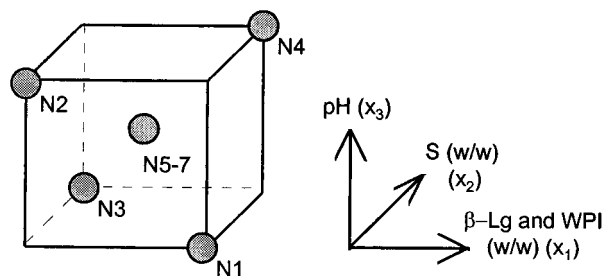


Figure 1. Scheme of the reduced, three-level, factorial design.

for reducing the dimensionality of multivariate data while preserving most of the variance, and it is therefore an excellent technique for observing the natural "patterns" in a data set. There are two kinds of patterns: relationships between rows in the data set and relationships between columns (Wold et al., 1984). The patterns are visualized by calculating principal components, whose function is to maximize the variance and covariance of a data set and which then can be divided into a complementary set of score and loading plots (Wold et al., 1987). Partial least-squares (PLS) was used to generate a set of coefficients, which describe the influences of the variables on the responses. PLS is a regression method by which quantitative relations can be established between the designed data block and the block of measured data (Carlsson, 1992). PLS deals with the responses simultaneously, taking their covariance into account, and it provides a coefficient overview plot. The number of significant coefficients used in the models is cross-validated.

The following response surface model, which accounts for the main effects, the two-factor interactions, and the three-factor interaction, was fitted with PLS regression to each measured response:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3 + \epsilon \quad (1)$$

In eq 1 y is the dependent measured response, β values are the regression coefficients, x_{1-3} are the independent variables, and ϵ is the overall error.

The model performance was validated by studying explained variance (R^2) and predictive capacity (Q^2), where R^2 describes the fraction of variation of the response explained by the model and Q^2 describes the fraction of the response that can be predicted by the model. Furthermore, the residuals for each response were analyzed by studying the normal probability plot of the residuals as well as the plot of observed values against the predicted values. The residuals are the difference between the model and the data set and also indicate model performance. All statistical calculations were performed using the software Modde 3.0 (Umetri AB, Umeå, Sweden).

RESULTS AND DISCUSSION

The results from the mechanical and barrier testing of films produced from the two different film materials, β -Lg and WPI, are presented side by side in this study. First, the multivariate analysis and statistical evaluation of these two model systems are presented. The model performance, the scattering of the experimental data, the variables that are correlated to the different responses, and the influence of the different variables on the responses are shown. Second, the quantitative and multivariate analyses of the results of the mechanical tests are presented. The multivariate analysis is divided into main and interaction effects, and every significant effect, as identified by the multivariate analysis and statistical evaluation, is presented and discussed. Finally, the quantitative and multivariate analyses of the barrier properties, MC and WVP, are

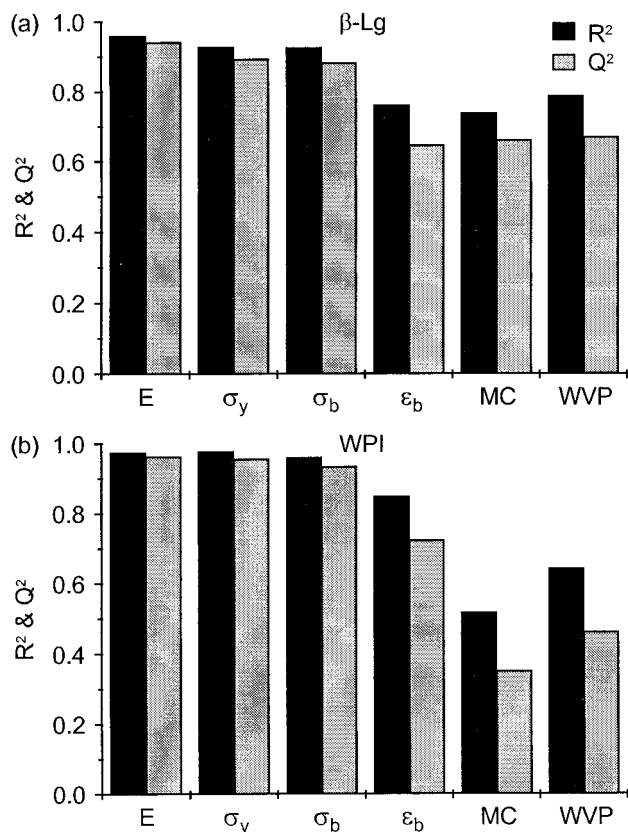


Figure 2. Summary of fit plot displaying R^2 and Q^2 values of β -Lg (a) and WPI (b) films as a function of every fitted response.

shown. Here too the multivariate analysis is divided into main and interaction effects.

The main effect plots displayed show the predicted change in the response when one variable varies from its lowest to its highest level, all other variables in the design being set to their average. Accordingly, the predicted change in the response for the interaction plots, when one variable varies from its lowest to its highest level, is plotted for both levels of the other variable, the remaining variable in the design being set to its average.

Multivariate Analysis and Statistical Evaluation. *Model Performance.* The model performance of the β -Lg and WPI films is shown in Figure 2. The responses from the mechanical tests (E , σ_y , σ_b) display very high values, close to 1, for both R^2 and Q^2 , which indicate very good models with good predictive power. The model performance for the ϵ_b of β -Lg and WPI films and the MC and WVP of β -Lg films is also good. The model performance for the MC and WVP of the WPI films is not as good as previous ones, but it models within the desired limits. A possible explanation for this is the small variation of the data from MC and some minor problems with the procedure for WVP measurement. The normal probability plot of the residuals for each response shows that the residuals are normally distributed and follow the normal probability line closely. The plot of observed values against the predicted values verifies the result displayed in Figure 2. The data points follow, closely, a straight line for the good models, whereas the data points are a little more scattered for the MC and WVP of the WPI films. Hence, the correlation coefficient r^2 varies between 0.99 and 0.96 for E , σ_y , and σ_b ; between 0.92 and 0.88 for ϵ_b ;

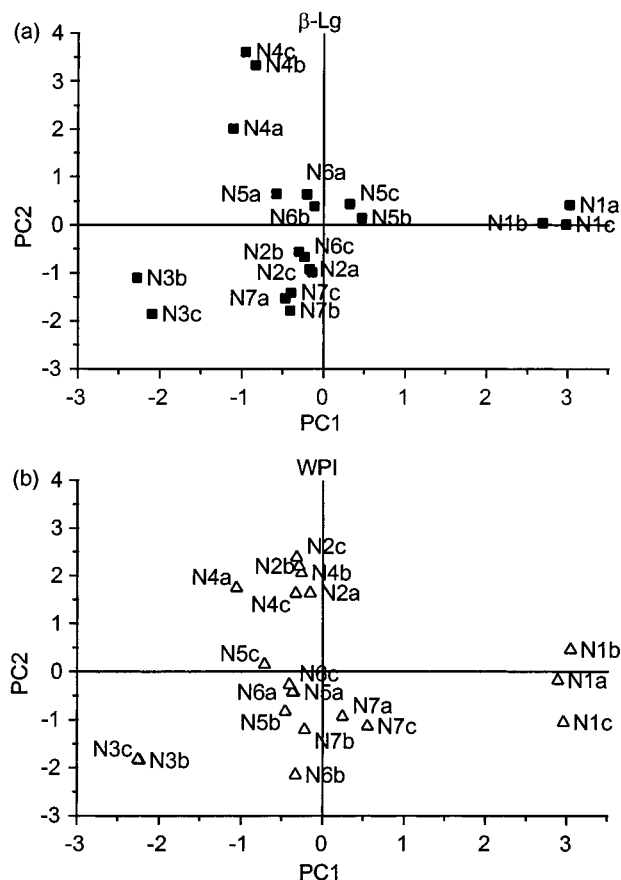


Figure 3. Score plots of β -Lg (a) and WPI (b) films of the first two PC vectors, PC1 and PC2.

between 0.86 and 0.89 for MC and WVP for the β -Lg films; and thus between 0.72 and 0.80 for the WPI films.

Scattering of the Experimental Data. In Figure 3, the scattering of the experimental data is displayed as score plots of the different design points with all replicates along principal components (PC) 1 and 2. PCA was run on the response data set, and three PC vectors were calculated. However, plotting the first two PC vectors against one another explains most of the systematic variation of the response data set. Replicates of the central design point, N5a-c, N6a-c, and N7a-c, are situated close to one another, which indicates good reproduction of the film-forming process in the experimental domain investigated. Design points that have little resemblance are situated far apart along the PC, for example, N1a-c versus N3b-c. This is not surprising since design points N1 and N3 have large differences in concentration of β -Lg, WPI, and sorbitol, which is used as a plasticizer. Design point N3a was an outlier in both the β -Lg and WPI data sets and has therefore been excluded as it reduces the predictive power of the response surface models. The probable cause for the outlier in both the β -Lg and WPI data sets is irregular WVP measurement data.

Variables That Are Correlated to the Responses. The loading plots shown in Figure 4 display the variables that are correlated to the different responses and consequently influence the responses. Again, the first and second PCs are plotted against one another. It can be seen that the responses E and WVP are negatively correlated, since they are plotted relatively close to a value 0.0 of PC2 and opposite each other with respect to origin. The loadings of variables plotted close to E

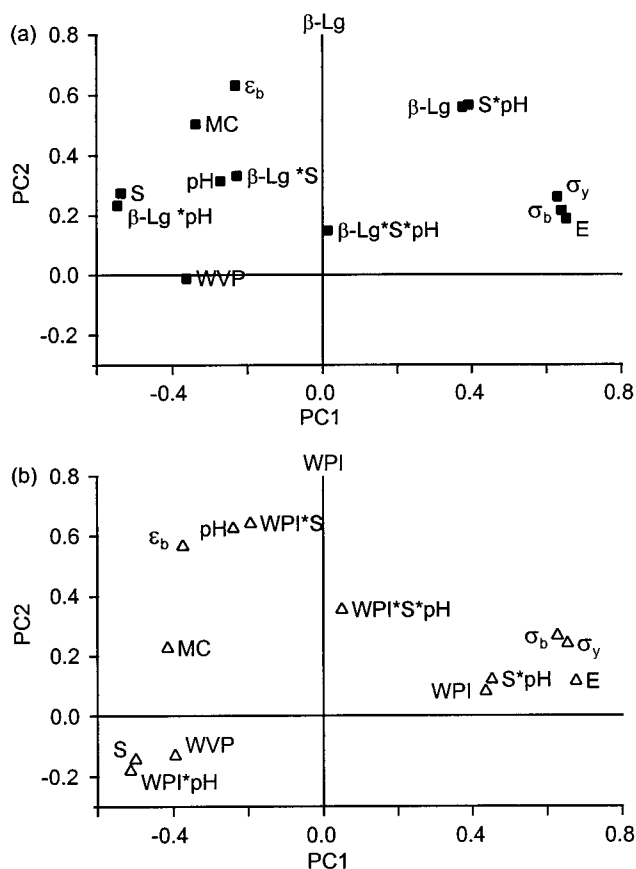


Figure 4. Loading plots of β -Lg (a) and WPI (b) films of the first two PC vectors, PC1 and PC2.

and WVP, respectively, are correlated with these responses, whereas loadings of variables plotted opposite to E and WVP are negatively correlated. For example, E values for the WPI films are positively correlated with the variable WPI and negatively correlated with the variable S (Figure 4b). This means that E is increased if the concentration of WPI is increased. Accordingly, E is decreased if the concentration of S is increased. This is the same result that also can be seen in the main effect plot on Young's modulus in Figure 7. The loading of variables that contribute to several responses is plotted in the quadrants of the loading plots (Carlsson, 1992).

Influence of the Different Variables on the Responses. Using PLS regression, six response surface models (eq 1) for both film materials were fitted to determine the influence of the different variables on the responses. In Figure 5, the β -coefficient value of every variable in the models is presented. Each model is statistically significant at the 95% confidence level. The β -coefficient is a relative measure of the influence of the corresponding variable on the response. A large main β -coefficient indicates that, within the experimental domain studied, a change in the corresponding variable leads to variation in the response, and this effect is independent of the other variables. For example, the β -coefficient of concentration S has a large influence on the WVP of β -Lg films, whereas the β -coefficient of pH is of minor importance (Figure 5a). Accordingly, a large interaction β -coefficient indicates that two variables cooperate in influencing the response. Hence, a value of a main or interaction β -coefficient equal to, or close to, zero would imply that the corresponding variable(s) is (are) not influencing the response.

Mechanical Properties. Quantitative Analysis of the Mechanical Properties. Four mechanical properties were derived from the tensile test: Young's modulus (E), stress at maximum force (σ_y), stress at break (σ_b), and strain at break (ϵ_b). The behavior of σ_y is almost identical with σ_b , and due to the fact that σ_b is more common in the literature, only the result from the latter is presented in this paper. The minor difference is that σ_y has slightly higher stress values owing to the nature of the stress and strain curves presented in Figure 6. Figure 6 is of vital interest because it clearly shows similarities and differences in the rheological behavior between the two film-forming materials. One should bear in mind the difference between the two materials in protein composition, as shown in Table 1. β -Lg is a purified material with >95% of the dominating protein β -lactoglobulin, whereas WPI is a commercial product that contains only 74% β -lactoglobulin.

The films produced at design point N1 are stiff and have a high stress and a low strain at break compared with the other films produced at the other design points. N1 consists of 14% (w/w) β -Lg or WPI and 10% (w/w) S at pH 7, as displayed in Table 2. Almost the opposite is true of the films cast at design point N3, which are weak but slightly more stretchable. These films also had some difficulty in sticking together due to their lack of solidity. N3 consists of 10% (w/w) β -Lg or WPI and 14% (w/w) S at pH 7. Furthermore, the central design points (N5–N7) display similar behavior, which indicates good reproduction of the film-forming process. The strain at break is almost the same for the β -Lg and WPI films, with ϵ_b values of 38 and 46%, respectively. The films produced at design point N4 reveal good mechanical properties with a strain at break of \sim 80%. Another interesting feature is the slightly different curvature between the WPI and β -Lg films.

Another aspect to take into consideration is the critical gel concentration (c_g) for β -lactoglobulin in solution, which varies with the pH (Stading and Hermansson, 1990). At pH 7, 8, and 9, c_g is approximately 10.5, 12, and 13.5% (w/w) β -lactoglobulin, respectively. The high c_g at pH 9 may indicate an alkali denaturation that disturbs the fine-stranded network formation. In Figure 6a, design point N2 with 10% (w/w) β -Lg and 10% (w/w) S at pH 9 is under c_g but still shows stresses similar to the ones found at the central design points N5–N7 for the β -Lg films even if the strain at break values are different. This may suggest that it is not necessary to reach c_g to obtain films with appropriate mechanical properties because of the more efficient concentration during drying. Hence, how c_g affects the film properties could be an interesting topic for future studies.

Multivariate Analysis of Mechanical Properties. Main Effects of the Concentration of β -Lg and WPI, Concentration of S, and pH. In Figure 7, the plot shows how E increases and decreases when the concentration of β -Lg and WPI and the concentration of S increase. E is lowered from approximately 48 to 27 MPa when the plasticizer concentration is increased. This is in agreement with the findings of McHugh and Krochta (1994a), who noted a decrease in E from 1000 to 450 MPa when the ratio WPI:S changed from 2.3:1 to 1:1. In the same study, ϵ_b increased from 1.6 to 8.7% when the ratio WPI:S changed from 2.3:1 to 1:1.

Figure 8 displays how σ_b changes when the β -Lg, WPI, and S concentrations vary. The behavior of σ_b follows

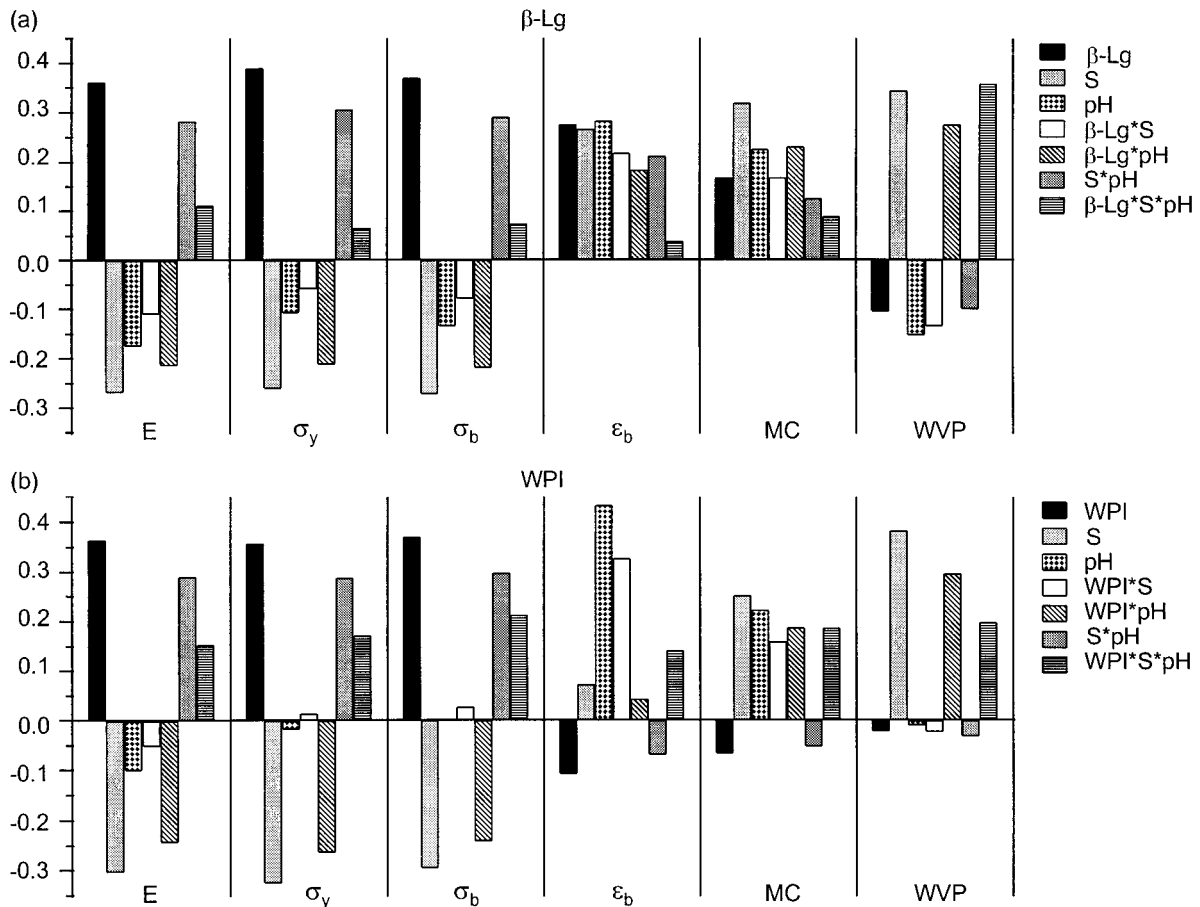


Figure 5. Normalized coefficient overview plot of β -Lg (a) and WPI (b) films as a function of every fitted response. The normalized coefficients are divided by the standard deviation of their respective response to make the coefficients comparable.

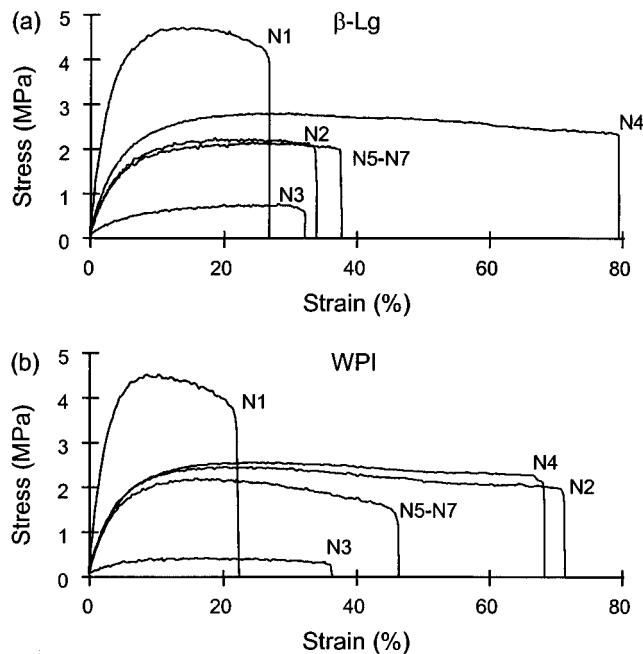


Figure 6. Tensile test plot displaying average stress and strain curves of β -Lg (a) and WPI (b) films for design points N1–N7.

the result presented above for E . When the protein concentration and concentration of S increase, σ_b increases and decreases, respectively. In the present study, design point N1 with the ratio WPI:S 1.4:1 shows quantitative σ_b and ϵ_b values of 3.5 MPa and 21.7%,

respectively. Fairley et al. (1996) presented similar results for the ratio WPI:S 3:1 coplasticized with small amounts of sodium dodecyl sulfate ($\sigma_b = 4.5$ MPa, $\epsilon_b = 21.2\%$). However, the plasticizing effect is a well-known phenomenon. Generally, increasing the amount of plasticizer weakens the intermolecular forces between adjacent polymer chains. This results in films with lower tensile strength and greater elongation (Gennadios et al., 1994a).

How an increase in pH affects the increase in ϵ_b is shown in Figure 9. The effect is more pronounced for the WPI films than for the β -Lg films, which can also be seen in Figure 6. The WPI films show a higher strain at break for design points N4 and N2 at pH 9 than for N3 and N1 at pH 7. The ratio of WPI:S is different between N4 and N2 versus N3 and N1, but the results nevertheless reveal a clear difference between pH 7 and 9. No information on how the mechanical properties are influenced when the pH is changed in β -Lg or WPI films has been found in the literature.

Interaction Effects of the Concentrations of β -Lg and WPI, Concentration of S , and pH. In Figures 10 and 11, the interaction effect plots on E and σ_b show two different types of behavior at pH 7 and 9. At pH 9, E and σ_b are not affected when the concentration of β -Lg, WPI, or S varies. However, at pH 7, E and σ_b increase when the concentration of β -Lg and WPI increases, and E and σ_b decrease when the concentration of S increases. This is the same result as presented for the main effects of E and σ_b in Figures 7 and 8, respectively, when the pH was set at 8. Hence, this difference in performance is probably due to a structural difference between the

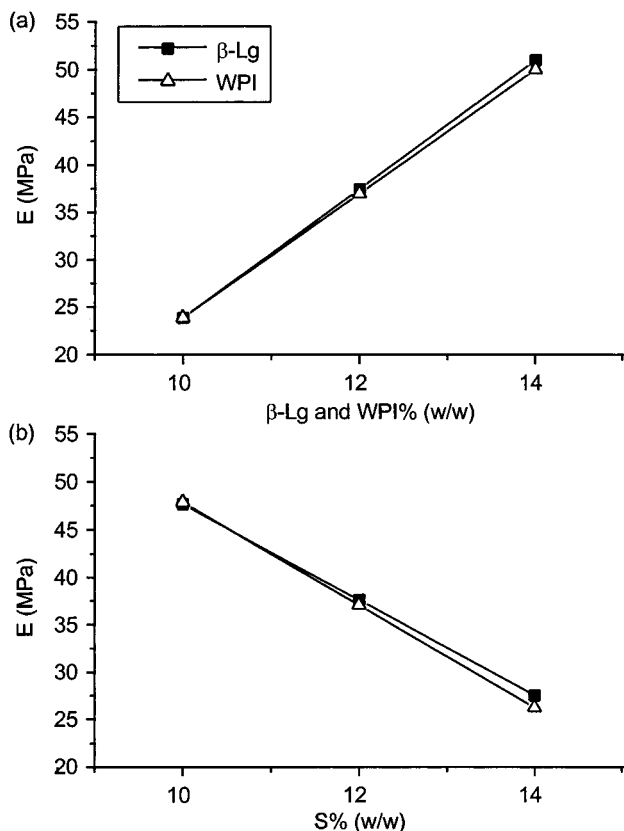


Figure 7. Main effect on Young's modulus (E) of β -Lg and WPI films as a function of (a) β -Lg and WPI concentrations [fixed variables: pH 8 and 12% (w/w) S] and (b) S concentration [fixed variables: pH 8 and 12% (w/w) β -Lg and WPI].

films at the different pH values, which need to be further studied.

Understanding the behavior of the film-forming solutions when the concentration of β -Lg and WPI changes and different pH values are applied is not easy. The amounts and different types of proteins vary between β -Lg and WPI and so, consequently, do the number of disulfide bonds and free thiol groups that may influence the globular structures. Thiol/disulfide exchange reactions, leading to formation of intermolecular disulfide bonds, are believed to play a role in the heat-induced aggregation and gelation of β -lactoglobulin, as reviewed by Hoffman (1997). Still, the extent of their relative contribution to the overall aggregation and gelation process is unclear (McSwiney et al. 1994).

Barrier Properties. *Quantitative Analysis of MC and WVP.* The MC and WVP in the β -Lg and WPI films were evaluated, and the quantitative result is presented in Figure 12. A clear distinction is that the MC is higher for the β -Lg films at each design point, and this is probably one reason for the higher WVP values (Figure 12b). This is in accordance with the results of Maté and Krochta (1996), who reviewed that WVP increases with increased MC for hydrophilic films, which was due to the plasticizing effect of the water. The theory behind this fact is that the water molecules disrupt polymer chain hydrogen bonding and cause an increase in the interstices, and thereby water vapor diffuses more rapidly. This is similar to what happens when the RH increases, which has been studied by numerous researchers. For example, McHugh and Krochta (1994b) showed the exponential effect of the WVP values when the RH increased. However, Maté

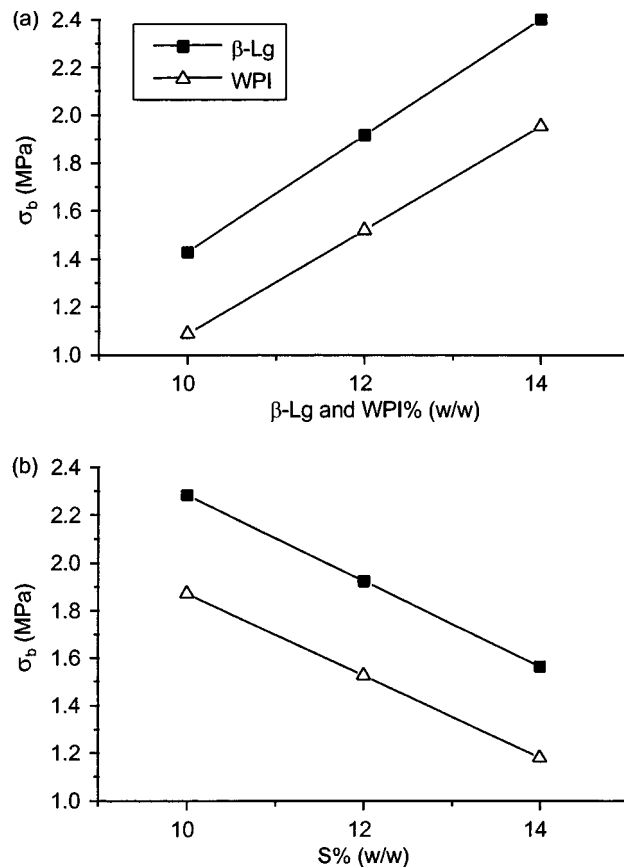


Figure 8. Main effect on stress at break (σ_b) of β -Lg and WPI films as a function of (a) β -Lg and WPI concentrations [fixed variables: pH 8 and 12% (w/w) S] and (b) S concentration [fixed variables: pH 8 and 12% (w/w) β -Lg and WPI].

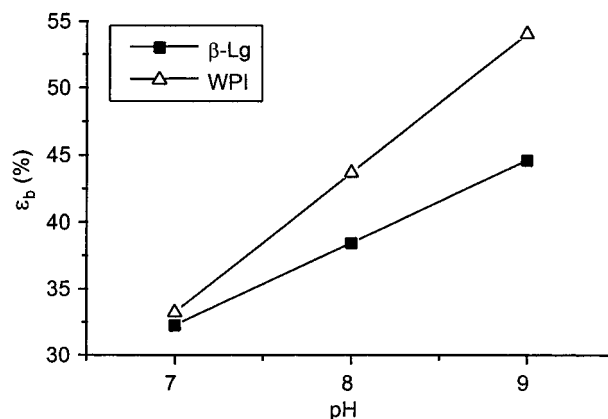


Figure 9. Main effect on strain at break (ϵ_b) of β -Lg and WPI films as a function of pH [fixed variables: 12% (w/w) β -Lg and WPI and 12% (w/w) S].

and Krochta (1996) stated that, for the two different WPIs examined, one with a high β -Lg content, the difference in WVP and oxygen permeability was not statistically significant.

The difference in MC and WVP between design points N3 and N4 for the β -Lg films is probably due to the change in concentration of the β -Lg, which likely effects the density of the polymer matrix. At design points N3 and N4 the concentration of S is 14% (w/w), whereas the concentrations of β -Lg are 10 and 14% (w/w), respectively. The higher total concentration of β -Lg and S at design point N4 probably increases the ability to gain and store moisture in the matrix compared to

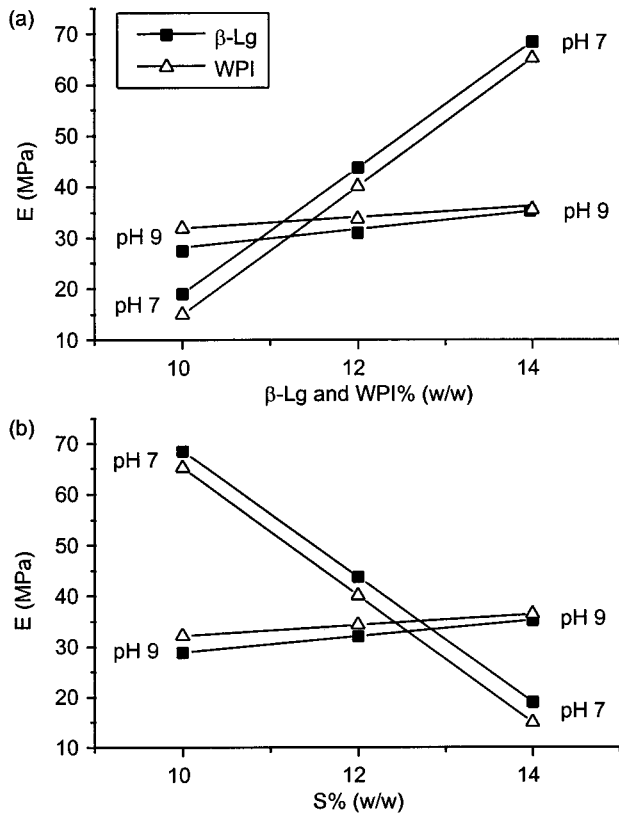


Figure 10. Interaction effect on Young's modulus (E) of β -Lg and WPI films as a function of (a) β -Lg and WPI concentrations and pH [fixed variable: 12% (w/w) S] and (b) S concentration and pH [fixed variables: 12% (w/w) β -Lg and WPI].

design point N3 (Figure 12a). Furthermore, design point N4 probably also has a more dense matrix, which improves the barrier against water vapor (Figure 12b).

Multivariate Analysis of MC and WVP. *Main Effect of the Concentration of S.* In Figure 13a, the plot shows the main effect of how MC increases when the concentration of S increases. This effect is probably due to the six hydroxyl groups that S contains, which can readily bond with water molecules and increase the water-holding capacity of the β -Lg and WPI films. Accordingly, in Figure 13b, the plot presents how the WVP increases when the concentration of S increases. Banker (1966) stated that when the amount of plasticizer increases, the interchain attractive forces are lowered, which reduces the energy of activation for diffusion (E_d). When E_d decreases, the diffusion constant increases and results in an increased permeability of water vapor and other gases.

The experimental conditions are a very important parameter, since it is a well-known fact that they influence the results. Therefore, all WVP values should be accompanied by data on the testing environment (Greener Donhowe and Fennema, 1994). Films should also be tested under conditions of temperature and RH that will be encountered in practice to avoid misleading results. Furthermore, a high RH gradient was in the past generally regarded as "worst case" data, since exposure to a smaller RH gradient was assumed to be a less severe condition. However, recent studies have shown that a smaller gradient at a more efficient condition, for example, 65–95% RH, represents a more severe test condition (Greener and Fennema, 1989). This fact can also be found in older literature: Rankin et al. (1958) showed that a 29–81% RH gradient gave higher

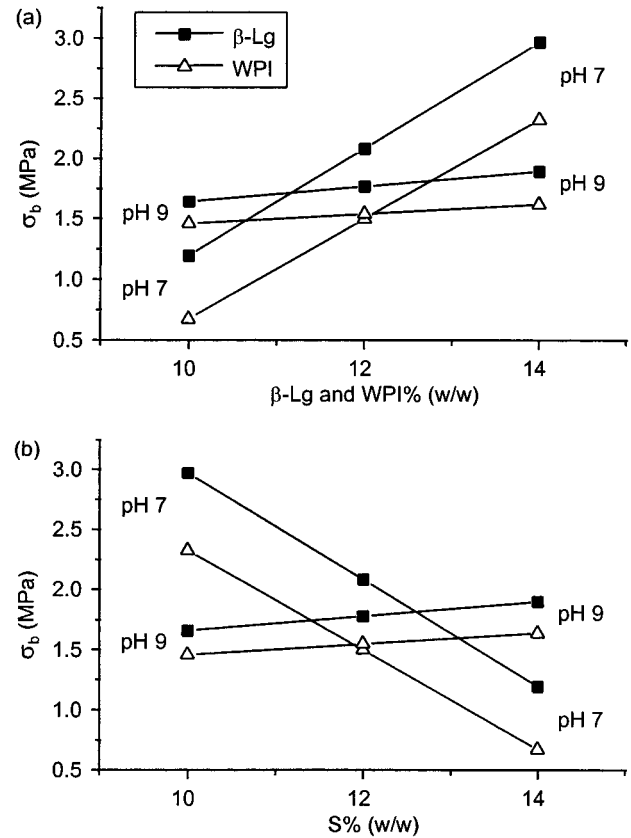


Figure 11. Interaction effect on stress at break (σ_b) of β -Lg and WPI films as a function of (a) β -Lg and WPI concentrations and pH [fixed variable: 12% (w/w) S] and (b) S concentration and pH [fixed variables: 12% (w/w) β -Lg and WPI].

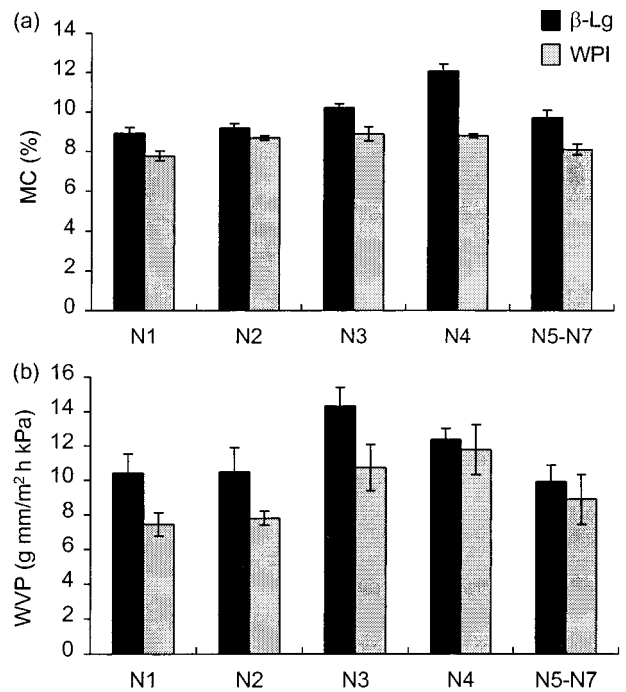


Figure 12. MC and WVP of β -Lg and WPI films for design points N1–N7. Error bars show standard deviation.

WVP values compared to a 1–53% RH gradient. This can further be exemplified with design point N2 (WPI:S 1:1), which showed a WVP value of $7.8 \text{ g}\cdot\text{mm}/\text{m}^2\cdot\text{h}\cdot\text{kPa}$. Experimental conditions were 23°C with a 50–72% RH gradient. McHugh and Krochta (1994c) tested films under less severe conditions, 25°C and 0–75% RH

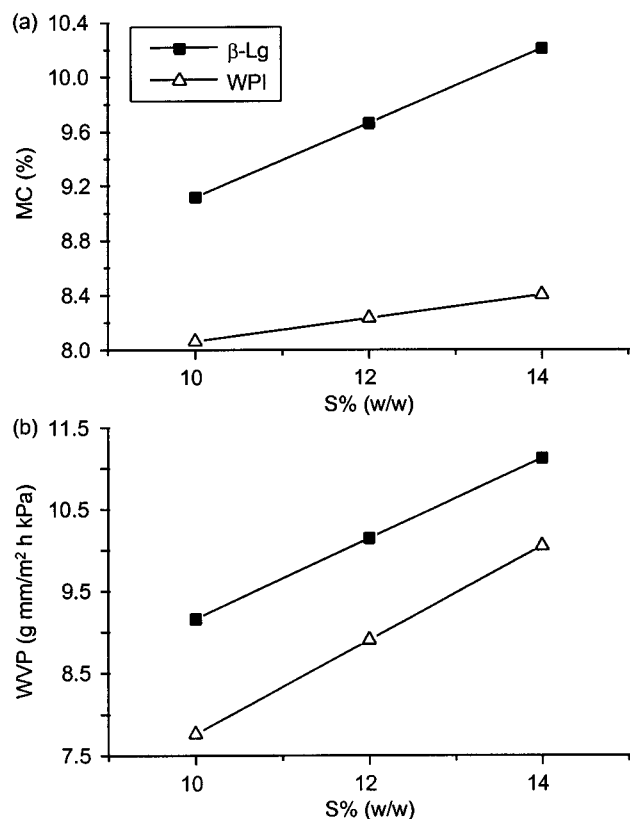


Figure 13. Main effect on MC and WVP of β -Lg and WPI films as a function of S concentration [fixed variables: pH 8 and 12% (w/w) β -Lg and WPI].

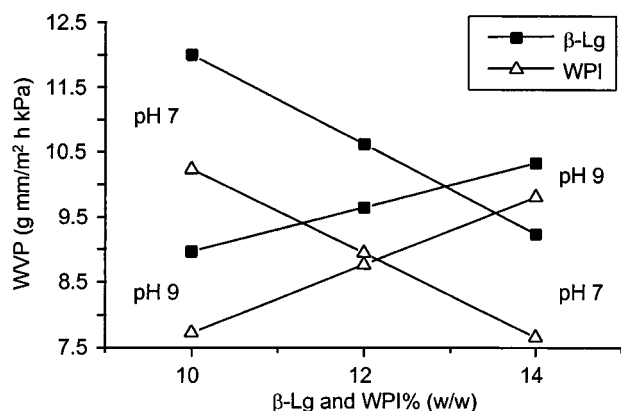


Figure 14. Interaction effect on WVP of β -Lg and WPI films as a function of β -Lg and WPI concentration and pH [fixed variable: 12% (w/w) S].

gradient, and consequently presented lower values of 3.5 g·mm/m²·h·kPa for a WPI:S ratio of 1:1.

Interaction Effect of the Concentration of β -Lg and WPI and pH. In Figure 14, the interaction effect plots on WVP show two different types of behavior at pH 7 and 9 when the concentrations of β -Lg and WPI vary. As the concentration of β -Lg and WPI increases, the WVP decreases and increases for pH 7 and 9, respectively. Another reflection is that the lowest WVP values are at pH 9 with 10% (w/w) β -Lg and WPI and at pH 7 with 14% (w/w) for both materials. This contrast in behavior at the different pH values is probably also due to a structural change in the polymer matrix previously discussed.

McHugh et al. (1994) presented results for a 10% (w/w) WPI with 6% (w/w) S films at different pH values.

The WVP values were, before denaturation, 2.71, 2.68, and 2.79 g·mm/m²·h·kPa for pH 7, 8, and 9, respectively. The test conditions were 25 °C and a 0–77% RH gradient. However, Figure 14 clearly shows a difference in WVP values between pH 7 and 9 for 10% (w/w) β -Lg and WPI films. Experimental conditions were 23 °C with a 50–72% RH gradient.

The results presented above, comparisons with other studies, and comparisons in general must be approached with caution due to different material suppliers and, hence, the possibility of differing materials, different molecular weights, test conditions, casting techniques, etc. Nevertheless, the results produced in this study were comparable with those produced by other researchers. β -Lg and WPI films are highly polar polymers, similar to many other proteins and polysaccharides films, resulting in high WVP values. On the other hand, the chemical characteristics that lead to high permeability for water vapor result in extremely low permeability for unpoler gases, for example, oxygen. This property is perhaps the most important feature of β -Lg and WPI films, when one is looking at new packaging materials for future use in the food industry. The interest from the packaging industry is also growing as whey protein films are edible and biodegradable. Furthermore, the barrier against water vapor can be improved by the addition of lipids. This can be accomplished by laminating an edible film with a lipid layer, by forming a composite film in which both hydrophilic and hydrophobic components are dispersed in a solvent and then dried, or by emulsifying the lipid within the hydrophilic phase (Shellhammer and Krochta, 1997).

Conclusions. The mechanical and barrier properties are greatly influenced by the ratio of the β -Lg and WPI concentrations and the S concentration. The two film-forming materials, β -Lg and WPI, show similar results, which can be attributed to the dominating protein β -lactoglobulin. This was in agreement with the findings of Langton and Hermansson (1992), who showed that the microstructure of whey protein gels at different pH values showed close resemblance to that of β -lactoglobulin gels at comparable pH values. pH exerts a strong effect on the different properties, and a structural difference in the polymer matrix occurs above pH 8. The multivariate analysis has proved to be a valuable tool for evaluating and quantifying the influences of the separate variables, as well as the interaction effects between the variables, in a specified experimental domain. Further research should be carried out with either β -Lg or WPI films to fully understand the changes in structural behavior at the different pH values. Microscopic techniques could be used to further study the structural behavior at different pH values presented in this work as well as dynamic mechanical analyses and differential scanning calorimetry for the thermal behavior.

ACKNOWLEDGMENT

Stable Micro System (Goldaming, England) is thanked for the loan of TA-XT2 texture analyzer.

LITERATURE CITED

Anker, M. *Edible and Biodegradable Films and Coatings for Food Packaging—A Literature Review*, SIK—The Swedish Institute for Food and Biotechnology, and Chalmers University of Technology, Department of Food Science: Göteborg, Sweden, 1996.

- ASTM. Standard Test Methods for Water Vapor Transmission of Materials. Designation E96-90. In *Annual Book of ASTM Standards*; ASTM: Philadelphia, PA, 1990; pp 834–841.
- ASTM. Standard Test Methods for Tensile Properties of Thin Plastic Sheeting. Designation D882-91. In *Annual Book of ASTM Standards*; ASTM: Philadelphia, PA, 1991; pp 182–190.
- ASTM. Standard Test Methods for Moisture Content of Paper and Paperboard by Oven Drying. Designation D644-94. In *Annual Book of ASTM Standards*; ASTM: Philadelphia, PA, 1994; pp 1–2.
- Banker, G. S. Film Coating Theory and Practice. *J. Pharm. Sci.* **1966**, *55*, 81–89.
- Carlsson, R. In *Design and Optimization in Organic Synthesis*; Vandeginste, B. G. M., Kvalheim, O. M., Eds.; Elsevier Science Publisher: Amsterdam, 1992.
- Chen, H. Functional Properties and Applications of Edible Films Made of Milk Proteins. *J. Dairy Sci.* **1995**, *78*, 2563–2583.
- Fairley, P.; Monahan F. J.; German, J. B.; Krochta, J. M. Mechanical Properties and Water Vapor Permeability of Edible Films from Whey Protein Isolate and Sodium Dodecyl Sulfate. *J. Agric. Food Chem.* **1996**, *44*, 438–443.
- Gennadios, A.; McHugh, T. H.; Weller, C. L.; Krochta, J. M. Edible Coatings and Films Based on Proteins. In *Edible Coatings and Films to Improve Food Quality*; Krochta, J. M., Baldwin, E. A., Nisperos-Carriedo, M., Eds.; Technomic Publishing: Lancaster, PA, 1994a.
- Gennadios, A.; Weller, C. L.; Gooding, C. H. Measurement Errors in Water Vapor Permeability of Highly Permeable, Hydrophilic Edible Films. *J. Food Eng.* **1994b**, *21*, 395–409.
- Gennadios, A.; Hanna, M. A.; Kurth, L. B. Application of Edible Coatings on Meats, Poultry and Seafoods: A Review. *Lebensm.-Wiss. -Technol.* **1997**, *30*, 337–350.
- Greener, I. K.; Fennema, O. R. Barrier Properties and Surface Characteristics of Edible, Bilayer Films. *J. Food Sci.* **1989**, *54*, 1393–1399.
- Greener Donhowe, I. K.; Fennema, O. R. Edible Films and Coatings: Characteristics, Formation, Definitions, and Testing Methods. In *Edible Coatings and Films to Improve Food Quality*; Krochta, J. M., Baldwin, E. A., Nisperos-Carriedo, M., Eds.; Technomic Publishing: Lancaster, PA, 1994.
- Hegg, P.-O. Thermal Stability of β -Lactoglobulin as a Function of pH and the Relative Concentration of Sodium Dodecylsulfate. *Acta Agric. Scand.* **1980**, *30*, 401–404.
- Hermansson, A.-M. Aggregation and Denaturation Involved in Gel Formation. In *Functionality and Protein Structure*; Pour-El, A., Ed.; ACS Symposium Series 92; American Chemical Society: Washington, DC, 1979.
- Hermansson, A.-M. Water- and fatholding. In *Functional Properties of Food Macromolecules*; Mitchell, J. R., Leward, D. A., Eds.; Elsevier Applied Science Publishers: London, 1986.
- Hoffman, M. A. M. β -Lactoglobulin: denaturation and aggregation. Ph.D. Thesis, University of Utrecht, The Netherlands, 1997.
- Krochta, J. M.; De Moulder-Johnston, C. Edible and Biodegradable Polymer Films: Challenges and Opportunities. *Food Technol.* **1997**, *51*, 61–74.
- Krochta, J. M., Baldwin, E. A., Nisperos-Carriedo, M., Eds. *Edible Coatings and Films to Improve Food Quality*; Technomic Publishing: Lancaster, PA, 1994.
- Langton, M.; Hermansson, A.-M. Fine-stranded and particulate gels of β -lactoglobulin and whey protein at varying pH. *Food Hydrocolloids* **1992**, *5*, 523–539.
- Maté, J. L.; Krochta, J. M. Comparison of Oxygen and Water Vapor Permeabilities of Whey Protein Isolate and β -Lactoglobulin Edible Films. *J. Agric. Food Chem.* **1996**, *44*, 3001–3004.
- McHugh, T. H.; Krochta, J. M. Sorbitol- vs Glycerol-Plasticized Whey Protein Edible Films: Integrated Oxygen Permeability and Tensile Property Evaluation. *J. Agric. Food Chem.* **1994a**, *42*, 841–845.
- McHugh, T. H.; Krochta, J. M. Water Vapor Permeability Properties of Edible Whey Protein-Lipid Emulsion Films. *J. Am. Oil Chem. Soc.* **1994b**, *71*, 307–312.
- McHugh, T. H.; Krochta, J. M. Permeability Properties of Edible Films. In *Edible Coatings and Films to Improve Food Quality*; Krochta, J. M., Baldwin, E. A., Nisperos-Carriedo, M., Eds.; Technomic Publishing: Lancaster, PA, 1994c.
- McHugh, T. H.; Avena-Bustillos, R.; Krochta, J. M. Hydrophilic Edible Films: Modified Procedure for Water Vapor Permeability and Explanation of Thickness Effects. *J. Food Sci.* **1993**, *58*, 899–903.
- McHugh, T. H.; Aujard, J.-F.; Krochta, J. M. Plasticized Whey Protein Edible Films: Water Vapor Permeability Properties. *J. Food Sci.* **1994**, *59*, 416–419, 423.
- McSwiney, M.; Singh, H.; Campanella, O. H. Thermal aggregation and gelation of bovine β -lactoglobulin. *Food Hydrocolloids* **1994**, *8*, 441–453.
- MD Foods Ingredients. Nr. Vium, Videbaek, Denmark, 1997.
- Miller, K. S.; Krochta, J. M. Oxygen and aroma barrier properties of edible films: A review. *Trends Food Sci. Technol.* **1997**, *8*, 228–237.
- Rankin, J. C.; Wolff, I. A.; Davis, H. A.; Rist, C. E. Permeability of Amylose Film to Moisture Vapor, Selected Organic Vapours, and the Common Gases. *Ind. Eng. Chem.* **1958**, *3*, 120–123.
- Shellhammer, T. H.; Krochta, J. M. Whey Protein Emulsion Film Performance as Affected by Lipid Type and Amount. *J. Food Sci.* **1997**, *62*, 390–394.
- Stading, M.; Hermansson, A.-M. Viscoelastic behaviour of β -Lactoglobulin gel structures. *Food Hydrocolloids* **1990**, *4*, 121–135.
- Stading, M.; Hermansson, A.-M. Large deformation properties of β -lactoglobulin gel structures. *Food Hydrocolloids* **1991**, *5*, 339–352.
- Stading, M.; Langton, M.; Hermansson, A.-M. Inhomogeneous fine-stranded β -lactoglobulin gels. *Food Hydrocolloids* **1992**, *6*, 455–470.
- Stading, M.; Langton, M.; Hermansson, A.-M. Microstructure and rheological behaviour of particulate β -lactoglobulin gels. *Food Hydrocolloids* **1993**, *7*, 195–212.
- Wold, S.; Albano, C.; Dunn, W.; Edlund, U.; Esbensen, P.; Geladi, S.; Hellberg, S.; Johansson, E.; Lindberg, W.; Sjöström, M. Multivariate data analysis in chemistry. In *Chemometrics: Mathematics and Statistics in Chemistry*; Kowalski, B. R., Ed.; Reidel: Dordrecht, The Netherlands, 1984.
- Wold, S.; Esbensen, K.; Geladi, P. Principal Component Analysis. *Chemom. Intell. Lab. Syst.* **1987**, *2*, 37–52.

Received for review October 10, 1997. Revised manuscript received February 4, 1998. Accepted February 11, 1998. This work has been supported by the Swedish Board for Technical Development (NUTEK), as well as Arla FoU, Stora Corporate Research AB, and Tetra Pak R&D.

JF9708711