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Investigation into the Physical and Chemical Properties of Sodium Caseinate - Maltodextrin Glyco-Conjugates

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Abstract

Casein–maltodextrin glyco-conjugates were prepared using an economical, food-grade process based on the Amadori re-arrangement of the Maillard reaction. The resultant glyco-conjugates were slightly yellow in colour and the degree of discolouration was dependent on heating time. Formation of glyco-conjugates was demonstrated by determining the reduction of free amino-groups by the O-phthaldialdehyde (OPA) assay and sugar reducing ends by gas chromatography-mass spectrometry (GC-MS). Increases in molecular weight were monitored by SDS-polyacrylamide gel electrophoresis (PAGE) and were in agreement with those predicted for the conjugation of casein monomers with malto-oligosaccharides of average DP 7-10. 2D-Urea-SDS-PAGE demonstrated that both protein and saccharide components co-migrate, indicating that covalent bonds were formed upon heating. This resulted in increases in mass-to-charge ratio of the materials, which suggested decreases in pI. These observed chemical and physical changes were reconciled with previously documented improvements in emulsifying properties.

Keywords: caseinate; maltodextrin; Amadori; glyco-conjugate; available lysine; reducing ends; GC-MS; SDS-PAGE
1. Introduction

Proteins are widely used in the food and cosmetic industries as emulsifying agents due to their amphoteric nature \textit{i.e.} they contain both charged hydrophilic and hydrophobic (lipophilic) regions, which results in a lowering of the surface tension at the oil-water interface (Dickinson, 1993; Shepherd, \textit{et. al.}, 2000). As pH is decreased towards the iso-electric point of the protein (~ pH 5 in the case of casein) this emulsifying activity as reduced, and may be lost as charge and protein solubility are considerably decreased. Thus, many commercial protein emulsifiers including casein are rendered useless at low pH and hence their application in food systems, many of which are acidic, is severely limited (Shepherd, \textit{et. al.}, 2000; Song, \textit{et. al.}, 2002).

It has been speculated that the chemical modification of proteins via conjugation with polysaccharides may improve emulsifying properties, especially at low pH as the iso-electric point and solubility will be altered and molecular integrity maintained (Kato, \textit{et. al.}, 1989, 1992, 1993; Kato, 1996; Nakamura, \textit{et. al.}, 1994; Nagasawa, \textit{et. al.}, 1996; Handa and Kuroda, 1999; Hattori, \textit{et. al.}, 2000a, b; Shepherd, \textit{et. al.}, 2000; Song, \textit{et. al.}, 2002; Babiker, 2002; Mislovicová, \textit{et. al.}, 2002).

Previous work in this laboratory (Shepherd, \textit{et. al.}, 2000) has shown that sodium caseinate can be chemically linked with 1,4-linked polysaccharides (\textit{e.g.} maltodextrin) via the Amadori re-arrangement (see Fayle and Gerrard, 2002) without excessive post-Amadori Maillard reactions, to form novel emulsifiers with improved emulsifying activity and stability at low pH.

In this paper we explain this improved emulsifying effect in terms of the modified physical and chemical properties of the glycoconjugates \textit{e.g.} colour change, loss of available lysine, loss of reducing ends, increase in molecular weight, formation of material with both carbohydrate-like and protein-like properties, change in mass-to-charge ratio and changes in size-exclusion chromatography elution profiles.
2. Materials and Methods

2.1. Materials

Sodium caseinate (Alanate 180) manufactured from skimmed cows’ milk was obtained from New Zealand Dairy Board, (Wellington, New Zealand). It contained 91-93 % protein, 0.8 % fat, 0.1 % lactose, 3.6 % ash and 4.1 % moisture (data supplied by NZDB). Maltodextrin (Maltrin 100), obtained from NZ Starch Products Ltd (Auckland, New Zealand), and prepared by enzymic hydrolysis of corn-starch and was reported to have a DE (dextrose equivalence) of 10. β- and κ-Caseins were purchased from Sigma Chemical Co. (St. Louis, MO) and α-casein from ICN Biomedicals Inc. (Aurora, OH) and were used without further purification. SDS-PAGE marker proteins (Precision) were purchased from BioRad (Hercules, CA).

2.2. Preparation of conjugates

Sodium caseinate and maltodextrin were individually dissolved in distilled water at 5 % w/v. Aqueous mixtures of protein and polysaccharide (1:1 v/v) were freeze-dried and heated at 60 °C and 70 % relative humidity (RH) in a humidity cabinet (Contherm Scientific Ltd., Petone, New Zealand) for 2 or 4 days (see Table 1). Reactions were carried out in shallow glass dishes and were stirred intermittently.

2.3. Observations on colour and UV absorbance of conjugates

Samples were dissolved in distilled water at 25 g l\(^{-1}\) on a protein basis and the solution colour was estimated visually, and at 1.0 g l\(^{-1}\) (on a protein basis, together with 1 g l\(^{-1}\) Maltrin M100 as a control) and the absorbance measured at 280 nm.

2.4. Determination of degree of conjugation

Assuming conjugates are formed via the Amadori rearrangement of the Maillard reaction this should lead to the loss of available primary amino groups \(i.e.\) lysine (Kato, \textit{et. al.}, 1988; Matsuda, \textit{et. al.}, 1991; Rufian-Henares, \textit{et. al.}; 2002; Dinnella, \textit{et. al.}).
and sugar reducing ends. Thus, the degree of conjugation was estimated using a colorimetric assay to determine the change in free amino groups and a GC-MS technique to determine the change in reducing end glucose residues.

Free amino groups were measured with the O-phthaldialdehyde (OPA) procedure with β-mercaptoethanol as the thiol component, as described by Bertrand-Harb, et al. (1993) using L-leucine as a standard. The degree of conjugation was calculated from the loss in amino groups compared to unreacted controls.

Reducing sugars were measured by a GC-MS technique as described by Sims, et al. (2001) for determination of degree of polymerisation of glucose polymers. Briefly, reducing end glucose residues in samples (~200 mg) were first reduced to their respective alditols with 1.0 M NaBH₄ in 2.0 M ammonia (200 µl), and then following hydrolysis with 2.5 M trifluoroacetic acid (TFA, 200 µl) for 1 h at 120 °C were acetylated to give glucitol hexaacetate. Non-reducing glucose residues were not reduced and following hydrolysis were acetylated resulting in the formation of the α- and β- anomers of glucopyranose pentaacetate and glucofuranose pentaacetate. A residue involved in the Amadori rearrangement becomes a 1-amino-1-deoxy-D-fructose residue and is therefore not detected by the analysis technique employed here. The change in free reducing ends relative to unreacted maltodextrin (assumed to represent 100 % reducing ends) was calculated from the molar ratio of glucitol hexaacetate to total glucose acetates.

2.5. **SDS-polyacrylamide gel electrophoresis (PAGE)**

2.5.1. **One-Dimensional SDS-PAGE**

Samples of approximately 1.0 g l⁻¹ protein were dissolved in distilled water, reducing SDS sample buffer (containing DTT to break disulphide bonds) added and boiled for 5 min to ensure complete denaturation and reduction of disulphide bonds. Samples (10 µl) were run on a 12 % acrylamide resolving gel according to Laemmli (1970). The gel was stained for proteinaceous material with Coomassie blue R250 and compared with molecular weight markers (Precision, BioRad, Hercules, CA) and α-,
β- and κ-caseins as reference standards. Malto-oligosaccharides do not migrate under these conditions.

2.5.2. Two-Dimensional Urea-SDS-PAGE

1st Dimension
Electrophoresis was adapted from the method of Davies and Law (1977). Casein or casein glycoconjugates were dissolved (~5 g l\(^{-1}\)) in a reducing buffer containing 5 M urea. The samples (20 µl) were applied to a 10 % polyacrylamide resolving gel (100 x 80 x 0.75 mm) containing Tris HCl pH 8.8 and 5 M urea. Electrophoresis was carried out in Tris-glycine buffer (pH 8.2) at room temperature and a 25 mA constant current. Prior to sample application, gels were subjected to a pre-run of 10 mins at 25 mA to remove impurities.

2nd Dimension
A sample lane from the first dimension was excised and incubated for 10 minutes in 10 ml equilibration buffer (50 mM Tris pH 6.8, 6 M Urea, 30 % glycerol, 1% SDS and 30 mM DTT). The strip was removed and then placed in 10 ml alkylation buffer (50 mM Tris pH 6.8, 6 M Urea, 30 % glycerol, 1 % SDS, 65 mM iodoacetamide and 0.001 % bromophenol blue). The alkylated caseins were then separated in the 2nd dimension by SDS-PAGE on 12.5 % polyacrylamide (100 x 80 x 1.5 mm) and compared to molecular weight markers (Precision, BioRad, Hercules, CA). The gel was fixed with 35: 10: 55 (methanol: acetic acid: water) and then stained for carbohydrate material according to the procedure of Carlsson (1993). The Schiff’s stained gel was recorded (Alphaimager, Alpha Innotech, San Leandro, CA) and then the gel was stained for proteinaceous material with Coomassie blue R250.

2.6. Size-exclusion chromatography

Substrates (2.0 g l\(^{-1}\)) and conjugates (4.0 g l\(^{-1}\)) were dissolved in distilled water and chromatographed (100 µl) on TSK Gel 5000 PW\(_{XL}\) and TSK Gel 4000 PW\(_{XL}\) (Tosoh Corp., Tokyo, Japan) columns (300 x 7.8 mm) connected in series and eluted with distilled water at 0.7 ml min\(^{-1}\) at 20 °C. The eluant was detected with an absorbance
monitor (Waters 450, Waters Corp., Milford, MA) and a refractive index monitor (Waters 2410, Waters Corp., Milford, MA).
3. Results and Discussion

3.1. Conjugation of sodium caseinate and maltodextrin

The reaction of sodium caseinate with maltodextrin resulted in an increase in colour (estimated visually) with heating time (Figure 1, Table 2) suggesting that some Maillard products were formed, despite the use of a O-4 substituted sugar which has been suggested to block the formation of these products (Shepherd, et. al., 2000). It is also possible that this was due to caramelisation of low molecular weight saccharides. Shepherd, et. al. (2000) showed dialysis of maltodextrin prior to heating reduced the discoloration of the resulting conjugates significantly suggesting that caramelisation was at least partly responsible for the colour of the conjugates.

The UV absorbance of samples also increased with heating time, for example the absorbance of SC1H4 is 1.18-fold greater that of SC on a g l⁻¹ protein basis (assumes SC1M, SC1FD, SC1H2 and SC1H4 are of 50 % protein content) - see Table 2. A similar increase in UV absorbance (at 310 nm) has been shown in the ribonuclease A - cyclotene system (Gerrard, et. al., 1999) and thought to be consistent with the formation of a Schiff base adduct between ribonuclease A and cyclotene, although this product was found to be unstable and to dimerise with the loss of two Schiff bases resulting in a subsequent decrease in UV absorbance.

The degree of conjugation of the sodium caseinate-maltodextrin product was determined by measuring the molar reduction in free amino groups and % reduction in sugar reducing ends (Table 2). Unreacted sodium caseinate was shown to contain 0.46 mmoles of free amino groups per gram. Sodium caseinate consists of approximately 40 % αs1-casein; 10 % αs2-casein; 40 % β-casein and 10 % κ-casein (see Morris, 2002 and references therein) and has a protein content of 92 % (see section 2.1). Thus, sodium caseinate can be calculated to contain approximately 7.5 wt. % lysine (see SWISS-Prot entries P02662, P02663, P02666 and P02668 for αs1-, αs2-, β- and κ-casein amino acid sequences, respectively - see also Morris, 2002), giving a theoretical value for free amino groups of 0.50 mmoles per gram, which is in good agreement abovevalue determined experimentally. A small reduction in free
amino groups was observed following freeze-drying of the sodium caseinate-
maltodextrin mixture, with a greater reduction upon heating at 60 °C for 2 days
(Table 2). Further heating (4 days) did not result in a further reduction in free amino
groups. This suggests that the remaining lysine residues are unavailable for reaction
under these conditions, and is consistent with our previous observations (Shepherd, et.
al., 2000) that there is only moderate improvement in emulsification activity over the
period of 1 to 4 days.

To compare the decrease in amino groups with that in sugar reducing ends it was
necessary to estimate the average molecular weight of the maltodextrin. Unreacted
maltodextrin gave a molar ratio of glucitol hexaacetate (derived from reducing end
residues) to glucose pentaacetate (derived from non-reducing residues) of 1:6.5,
giving an average degree of polymerisation (DP) of 7.5, which is equivalent to 0.81
mmoles of reducing ends per gram of maltodextrin. The DE of the maltodextrin was
reported by the supplier as 10, which from DE = 100/DP gives an average DP of 10
and hence is equivalent to 0.61 mmoles reducing ends per gram of maltodextrin.
Thus, the molar reduction in reducing ends was calculated using these two DP values
(Table 2). The results show that the reduction in amino groups on heating is mirrored
by an almost equal reduction in sugar reducing ends, consistent with the formation of
covalent links between the protein and polysaccharide. In fact, the expected 1:1
molar ratio reduction in reactive groups is found if the maltodextrin is assumed to
have an average DP of 9. We can also see that the reactive mixture contained
approximately 3 reducing ends for every 2 lysine residues, of which 38 % and 57 %
respectively have reacted.

If we take an average casein “monomer” molecular weight of 23,000 g mol\(^{-1}\), we can
then predict an increase in molar mass of 10,000 g mol\(^{-1}\), 13,000 g mol\(^{-1}\) and 12,000 g
mol\(^{-1}\) for conjugation of 57 % (8 out of 14) of the lysine residues with malto-
oligosaccharides of weight-average DP 7.5, 10 and 9, respectively. This does not take
into account the possible cross-linking of post-Amadori products and hence the
formation of larger molecular weight polymeric material (Fayle and Gerrard, 2002).
3.2. Analysis of conjugates by SDS-PAGE

3.2.1. One-Dimensional SDS-PAGE

The samples SC, SC1M and SC1FD which had not been heated all show an electrophoretic pattern typical of sodium caseinate, i.e. three bands at approximately 20,000 - 25,000 g mol\(^{-1}\) (Da) resulting from \(\alpha\), \(\beta\)- and \(\kappa\)-casein monomers (Figure 2). Heated samples SC1H2 and SC1H4 are marked by an absence of these distinct bands and the presence of a diffuse band over a larger molecular weight range although the bulk of material is in the range 25,000-37,000 g mol\(^{-1}\), which is within the expected spread of molecular weights taking into account the different molar masses and amounts of lysine in the individual caseins. The presence of higher molecular weight material indicates that some aggregation or cross-linking is occurring. These results are very similar to those of Shepherd, et. al. (2000), suggesting that conjugation and cross-linking has taken place resulting in total loss of “unmodified” material and the formation of higher molecular weight material. It has been demonstrated that heating of sodium caseinate alone results in insoluble aggregates, and that the presence of maltodextrin is required for improved solubility and emulsification activity (Shepherd, et. al., 2000).

3.2.2. Two-Dimensional Urea-SDS-PAGE

Caseins and casein glycoconjugates were separated on the basis of their mass-to-charge ratio, \(m/z\) in the first dimension (Fayle and Gerrard, 2002) using an alkaline-urea electrophoresis format and were then separated on the basis of molecular weight in the second dimension by SDS-PAGE.

In the case of SC1M (Gels A and B in Figure 3), it can be seen clearly that in the 1\(^{st}\) dimension there are two distinct bands and one more diffuse band and in the 2\(^{nd}\) dimension (Figure 3A) these bands migrate in the pattern typical of \(\alpha\)-, \(\beta\)- and \(\kappa\)-casein from left-to-right respectively, (see lanes 2 - 4 in Figure 2 for comparison). Furthermore there is little or no evidence of co-migrating carbohydrate material (Figure 3B) indicating that no conjugation has taken place as a result of mixing alone. As for SC1H4 (Gels C and D in Figure 3), in the 1\(^{st}\) dimension the bands are in
general more diffuse, however the differences are perhaps more pronounced in the 2\textsuperscript{nd} dimension where:

(i) the three protein bands have been shifted to higher molecular weights and higher mass-to-charge ratios (Figure 3C); and

(ii) it is now clear that carbohydrate material is co-migrating with protein material, a good indication of glyco-conjugation (Figure 3D).

As with Figure 2 the vast majority of the material is in the region of 5,000 - 15,000 g mol\(^{-1}\) greater than that of the native monomers, which is expected, although, cross-linked material is present as was the case in 1D-SDS-PAGE. It is therefore clear that heating sodium caseinate with maltodextrin for 4 days at 60 °C and 70 \% RH results in glycosylation of the protein and increase in molecular weight and mass-to-charge ratio, \(m/z\). An increase in \(m/z\) may be due to a decrease in protein charge due to an increased net negative charge on casein (O’Connell and Fox, 1999) and/or an increase in molecular weight. A decrease in charge and hence pI has previously been shown in the glycation of recombinant human serum albumin (rHSA) (Smales, et. al., 2002) using 2D-PAGE. An increase in molecular weight was also shown by electrospray ionisation mass spectrometry (EI-MS). Therefore an increase in \(m/z\) is consistent with the covalent bonding of lysine with carbohydrate.

3.3. Analysis of conjugates by size-exclusion chromatography

The total mass of material injected was \(2.0 \times 10^{-4}\) g for SC and M100 and \(4.0 \times 10^{-4}\) g for SC1M, SC1FD, SC1H2 and SC1H4 (i.e. \(2.0 \times 10^{-4}\) g protein and \(2.0 \times 10^{-4}\) g carbohydrate).

It appears from size-exclusion chromatography (SEC) that the maltodextrin-caseinate conjugates have hydrodynamic volumes similar to those of caseinate alone although there is evidence of conjugation at the high molar mass end of the elution profile (Figure 4). The difficulty in separating caseinate - maltodextrin glycoconjugates from unreacted substrates by SEC has been previously reported (Shepherd, et. al., 2000).
The small peak at the right of the UV chromatograms (in SC1H2 and SC1H4) was attributed to low molar mass protein degradation products formed upon heating, the proportion of which is greater after 4 days. In SC1M the peak at the right-hand side of the RI chromatogram (elution volume ~ 30 mins) shows no corresponding UV peak and is therefore considered to be of carbohydrate (M100) origin. Integration reveals that the area of this peak is reduced by approximately 30-35 % upon heating for 2 or 4 days at 60 ºC and 70 % RH consistent with covalent attachment of malto-oligosaccharides to the higher molecular weight caseins.

Upon analysis of the UV chromatograms it is clear that there is a considerable increase in UV absorbing material after conjugation. From the UV absorbance values shown in Table 2 we would expect increases in the total area under the UV chromatograms for SC1H2 and SC1H4 of 1.10 and 1.18-fold over that of SC, respectively. We find however increases of 1.40 and 1.53-fold. This constitutes an increase in protein and/or conjugate material of 27 and 29 % respectively in the cases SC1H2 and SC1H4. This increase in proteinaceous material was supported by a similar analysis of refractive index chromatograms. One explanation is that SEC columns retain some native caseinate and the covalent attachment of carbohydrate to caseinate results in a decrease in the hydrophobicity of the caseinate “sub-micelle” and hence a decrease in binding interactions with the column. Loss of lysine has been found previously to decrease sodium caseinate hydrophobicity (O’Connell and Fox, 1999). It has been shown for the protein legumin (Semenova, et. al., 1999) that the degree of polymerisation of covalently attached maltodextrins has varying effects on protein unfolding and hence hydrophilic/hydrophobic balance.

The differences in size-exclusion chromatograms are clearly seen in the difference SEC obtained by subtracting the profiles for SC1M from those of SC1H4 (Figure 5). The UV chromatogram is in general positive indicating the decrease in column interactions. There is an increase in larger molecular weight material upon conjugation together with a loss of low molecular weight carbohydrate material (see RI chromatogram) and an increase in low molecular weight protein material (see UV chromatogram).
Conclusions

We have found good chemical and physical evidence *i.e.* loss of available lysine (OPA assay), matching molar loss of reducing ends (GC-MS), increase in molecular weight (SDS-PAGE and 2D-Urea-SDS-PAGE), formation of material with both carbohydrate-like and protein-like properties (2D-Urea-SDS-PAGE) and change in mass-to-charge ratio (2D-Urea-SDS-PAGE) to indicate that sodium caseinate - maltodextrin glyco-conjugates have been formed via the Amadori rearrangement of the Maillard reaction (see Table 2)

The increases in molecular weights of casein “monomers” were within the predicted range of 10,000 – 13,000 g mol$^{-1}$ for the glyco-conjugation of a protein of average molecular weight 23,000 g mol$^{-1}$ with oligosaccharides of average DP 7-10 on 57% of the total lysine groups. The spread in molecular weights is due to the heterogeneity of casein and the polydispersity of the maltodextrin. This is further complicated in the “native” state (not denatured by SDS and urea) by concentration and structure dependent casein aggregation (Morris, 2002), which made it is impossible to determine molecular weights using size-exclusion chromatography.

These chemical and physical observations are further supported by colour changes, increases in UV absorbance and changes in the elution profiles as monitored by size exclusion chromatography with both refractive index and UV detection. Finally these glyco-conjugates appear to have increased solubility and therefore better emulsifying activity and emulsion stability at lower pHs (see Shepherd, *et. al.*, 2000).

It seems likely that covalent linking of a sugar moiety to the sodium caseinate submicelle has the effect of lowering the iso-electric point of the protein complex (Smales, *et. al.*, 2002), preliminary 2D-SDS-PAGE results support this (Gerrard personal communication) and hence increasing solubility (solubility is the determining factor in the ability of a protein/ protein complex to form an emulsion (Chevalier, *et. al.*, 2001)) at lower pH as has been described previously for Maillard products of ribonuclease A (Fayle, *et. al.*, 2001). Although the decrease in hydrophobic interactions of caseinate due to the loss of available lysine (O’Connell and Fox, 1999) and/ or the steric stabilisation effect of covalently attached
carbohydrate groups may also contribute to improved emulsifying stability (Akhtar and Dickinson, 2003).

Acknowledgements

Thanks to Dawn Scott and Keryn Johnson (Bioprocessing, Industrial Research Ltd.) for their help with SDS-PAGE and OPA assays. I would also like to thank Nigel Larsen, Graeme Coles, Jafar Al Hakkak, Kevin Sutton, (New Zealand Institute for Crop and Food Research Limited, Christchurch, New Zealand), and Juliet Gerrard (Dept. of Plant and Microbial Sciences, University of Canterbury) for their helpful discussions and suggestions. This work was supported by a New Zealand Foundation for Research, Science and Technology Contract.
References


Table 1 Sample nomenclature.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>sodium caseinate (Alanate 180)</td>
</tr>
<tr>
<td>M100</td>
<td>maltodextrin (Maltrin 100)</td>
</tr>
<tr>
<td>SC1M</td>
<td>1:1 mixture sodium caseinate and maltodextrin</td>
</tr>
<tr>
<td>SC1FD</td>
<td>1:1 mixture sodium caseinate and maltodextrin - freeze-dried</td>
</tr>
<tr>
<td>SC1H2</td>
<td>1:1 mixture sodium caseinate and maltodextrin - freeze-dried and heated for 2 days at 60 °C and RH 70 %</td>
</tr>
<tr>
<td>SC1H4</td>
<td>1:1 mixture sodium caseinate and maltodextrin - freeze-dried and heated for 4 days at 60 °C and RH 70 %</td>
</tr>
</tbody>
</table>
Table 2 Changes in colour, and UV absorption, and reduction in free amino groups and sugar reducing ends of sodium caseinate-maltodextrin conjugates with heating at 60 °C and 70 % RH. Results are normalised to an equivalent concentration of sodium caseinate or maltodextrin of 1 g l⁻¹.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Colour (visual estimate)</th>
<th>A₂₈₀ nm</th>
<th>-NH₂ (mmoles l⁻¹)</th>
<th>Reducing ends</th>
<th>mmoles l⁻¹ (DP~10) calculated from DE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(% of M100)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mmoles l⁻¹ (DP~7.5) from GC-MS</td>
<td></td>
</tr>
<tr>
<td>SC</td>
<td>clear (slightly turbid)</td>
<td>0.715</td>
<td>0.46</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>M100</td>
<td>clear</td>
<td>0.004</td>
<td>n/a</td>
<td>100</td>
<td>0.81</td>
</tr>
<tr>
<td>SC1M</td>
<td>clear (slightly turbid)</td>
<td>0.728</td>
<td>0.44</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SC1FD</td>
<td>clear (slightly turbid)</td>
<td>0.734</td>
<td>0.40</td>
<td>93.5</td>
<td>0.76</td>
</tr>
<tr>
<td>SC1H2</td>
<td>pale yellow</td>
<td>0.789</td>
<td>0.20</td>
<td>63.4</td>
<td>0.51</td>
</tr>
<tr>
<td>SC1H4</td>
<td>yellow</td>
<td>0.842</td>
<td>0.20</td>
<td>62.1</td>
<td>0.50</td>
</tr>
</tbody>
</table>
Legends to Figures

Figure 1 The effect of processing on the colour of sodium caseinate/maltodextrin mixtures. Samples from left to right SC1M, SC1FD, SC1H2 and SC1H4 at concentrations of 25 g l\(^{-1}\).

Figure 2 Coomassie-blue stained SDS gel. Lanes 1 - 10 (left - right) are of marker proteins, \(\alpha\)-casein, \(\beta\)-casein, \(\kappa\)-casein, SC, M100, SC1M, SC1FD, SC1H2 and SC1H4 respectively (conditions as described in section 2.5.1).

Figure 3 Representation of 2D-Urea-SDS gels: A - SC1M protein stain; B - SC1M carbohydrate stain; C - SC1H4 protein stain and D - SC1H4 carbohydrate stain (conditions as described in section 2.5.2). \(m/z\) increases from left-to-right.

Figure 4 Size-exclusion chromatograms for SC; M100; SC1M; SC1FD; SC1H2 and SC1H4 from both refractive index (RI) detector (thin line) and UV detector at 280 nm (thick line). Conditions as described in section 2.6. Void and total volumes are 12 and 33 minutes respectively. N.B. All chromatograms are to the same scale.

Figure 5 Size-exclusion chromatograms resulting from subtraction of the SC1M profiles from SC1H4 profiles from both refractive index (RI) detector (thin line) and UV detector at 280 nm (thick line).
Figure 2
Figure 3
Figure 4
Figure 5