ELSEVIER

Food Research International 36 (2003) 267–274

FOOD RESEARCH INTERNATIONAL

www.elsevier.com/locate/foodres

Effect of cross-linking with transglutaminase on the heat stability and some functional characteristics of sodium caseinate

J. Flanagan, Y. Gunning, R.J. FitzGerald*

Department of Life Sciences, University of Limerick, Limerick, Ireland

Received 29 April 2002; accepted 25 July 2002

Abstract

The effect of heating (140 °C, 0–60 min between pH 6.0 and 7.0) on the turbidity, pH 4.6 soluble amino group content and urea PAGE profiles of sodium caseinate (NaCN) and transglutaminase (TGase)-treated NaCN was determined. pH-dependent heat-induced changes in the turbidity and urea PAGE profiles of NaCN were initially attributed to casein aggregation followed by subsequent degradation on extended heating. Cross-linked NaCN samples (incubated with TGase at 20 °C, [E:S] of 1:50 and 1:20 for 185 min) were generally less turbid and had lower pH 4.6 soluble amino group content on heating than unmodified NaCN. The nitrogen solubility of cross-linked NaCN was improved at pH 2.0, 3.0 and 5.0. Some improvements in emulsifying activity index and stability of cross-linked NaCN were observed at pH 5.0 and 10.0. The improved heat stability and nitrogen solubility observed after TGase cross-linking may help extend the range of applications for NaCN.

Keywords: Sodium caseinate; Transglutaminase; Heat stability; Solubility

1. Introduction

The ability of transglutaminase (TGase; E.C. 2.3.2.13) to modify the functional properties of food proteins has been extensively reviewed (Kuraishi, Yamazaki, & Susa, 2001; Lorenzen, & Schlimme, 1998; Motoki & Seguro, 1998; Nielson, 1995; Zhu, Rinzema, Tramper, & Bol, 1995). By acyl group transfer between the ε-amino group of lysine and the γ-carboxyamide group of glutamine residues in proteins/peptides, TGase catalyses the formation of an ε-(γ-glutamyl)lysine isopeptide bond. In the absence of free ε-groups, water acts as the acyl acceptor, resulting in the deamidation of glutamine to glutamic acid.

The nitrogen solubility of individual caseins and sodium caseinate (NaCN) has been increased on incubation with a calcium-dependent TGase isolated from guinea pig liver (Motoki, Nio, & Takinami, 1984; Motoki, Seguro, Nio, & Takinami, 1986) and with a calcium-independent TGase isolated from a microbial source (Flanagan & FitzGerald, in press b; Nonaka, Sawa, Matsuura, Motoki, & Nio, 1996; Lorenzen, 2000). During a study on the effects of combined enzymatic hydrolysis and TGase-catalysed cross-linking of NaCN, Flanagan and FitzGerald, (2002a) reported that the solubility of NaCN was dependent upon the extent of cross-linking with TGase. Limited and extensive cross-linking resulted in significant improvements in solubility at low pH (pH 2.0 and 3.0). Improvements in solubility were also observed at pH 5.0 for the minimal and extensively cross-linked samples. The emulsifying, foaming and viscosity properties of these cross-linked samples were subsequently studied (Flanagan & FitzGerald, in press). In general, improvements in emulsifying activity index (EAI) were observed for the minimal, limited and extensively cross-linked samples at pH 5.0, 9.0 and 10.0, compared to unmodified NaCN. The extensively cross-linked sample exhibited improved emulsion stability at pH 5.0 compared to unmodified NaCN. Minimal and limited extents of cross-linking resulted in improved foam expansion at the extremes

* Corresponding author. Tel.: +353-61-202598; fax. +353-61-331490.

E-mail address: dick.fitzgerald@ul.ie (R.J. FitzGerald).

0963-9969/03/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0963-9969(02)00168-0

of pH, while extensive cross-linking yielded poor foamability.

Cross-linking NaCN with TGase after emulsion formation was reported to result in increased average droplet size (Dickinson & Yamamoto, 1996) while cross-linking prior to emulsion formation significantly reduced Ostwald ripening rates over long storage times (Dickinson, Ritzoulis, Yamamoto, & Logan, 1999). Although TGase-catalysed cross-linking of β-casein decreased emulsifying activity index (EAI), the storage stability of the emulsions increased with increasing extent of polymerisation (Liu & Damodaran, 1999). Lorenzen (2000) reported an increase in the stability and viscosity of emulsions prepared with cross-linked NaCN compared to non-cross-linked NaCN. However, cross-linked NaCN displayed decreased foam volume and stability compared to non-cross-linked NaCN. The inclusion of TGase in the formation of casein gels resulted in faster forming gels with higher viscoelastic moduli than those obtained by acidification or rennetting (Schorsch, Carrie, & Norton, 2000).

The effect of heating on the physicochemical and functional properties of milk (O'Connell & Fox, 2002; Singh & Creamer, 1992) and of NaCN (Guo, Fox, Flynn, & Kindstedt, 1996; Guo, Fox, Flynn, & Mohammad, 1989; Hustinx, Singh, & Fox, 1997; Jahaniaval, Kakuda, Abraham, & Marcone, 2000; Metwalli & van Boekel, 1998; van Boekel, 1999) has been extensively studied. However, apart from Lorenzen (2000) who observed a small decrease in heat stability on heating a TGase-catalysed cross-linked NaCN solution at 120 °C for <30 s, little information appears in the literature on the heat stability of TGase cross-linked NaCN.

The objective of this study was to characterise the effect of two different extents of cross-linking with TGase and heating at 140 °C for up to 60 min between pH 6.0 and 7.0 on the turbidity, pH 4.6 soluble amino group content and electrophoretic profiles of NaCN. Furthermore, the nitrogen solubility, emulsifying and foaming properties between pH 2.0 and 10.0 of the cross-linked NaCN samples were also determined.

2. Materials and methods

2.1. Materials

NaCN (85.35 g 100 g^{-1} protein) was supplied by Armor Protéines (Saint-Brice-en Coglès, France). Food-grade oil was purchased at local foodstores (Mazola™ Corn Oil, CPC (UK) Ltd., Esher, Surrey, UK). Glycine, trizma base, trichloroacetic acid (TCA), ammonium persulphate (APS), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), *N*, *N*′ methylene bis-acrylamide, and acrylamide (all electrophoretic grade), and α-, β- and κ-casein standards for urea PAGE, Sudan III, L-leucine and trinitrobenzene sulphonic acid (TNBS) were from Sigma Chemical Co. (Poole, Dorset, England). Kjeldahl catalyst tablets and urea (GPR grade) were from BDH (Leicestershire, England).

Calcium independent TGase from *Streptoverticillium* spp. was kindly supplied by Forum Products Ltd. (Brighton Road, Redhill, Surrey, England).

2.2. Preparation of cross-linked NaCN

Cross-linked NaCN samples, designated CL-a and CL-b, were prepared by incubating NaCN (4 g protein 100 ml^{-1}) at room temperature with TGase at 20 °C, pH 7.0 for 185 min at [E:S] ratios of 1:50 and 1:20 (g of enzyme preparation:g of protein substrate), respectively. The TGase reaction was inactivated by heating the sample at 80 °C for 1 min and 0.02 g 100 ml^{-1} sodium azide was added on cooling to prevent microbial growth. Solubility, emulsifying and foaming analyses were performed after sample generation and the remaining cross-linked and unmodified NaCN samples were stored at −18 °C prior to heat treatment studies.

2.3. Heat treatment

Cross-linked and unmodified NaCN samples (~4 g protein 100 ml^{-1}) were thawed at room temperature, adjusted to pH values between 6.0 and 7.0 using 0.1 N HCl or NaOH as required and made up to a final concentration of 1.0 g protein 100 ml^{-1} with distilled water. Aliquots (1.5 ml) of the samples were pipetted into 3 ml glass tubes (10 mm i.d.×120 mm, AGB Scientific, Dublin, Ireland), which were subsequently sealed with silicone bungs. The tubes were heated in a silicone oil bath (Elbanton BV, Kerkdriel, The Netherlands) at 140 °C, with continuous rocking at motor speed setting 4 for different times. The tubes were removed and immediately placed in ice water prior to turbidity and pH 4.6 soluble amino group content determination.

The heated samples were diluted 1:1 with distilled water prior to turbidity estimation at 400 nm. Turbidity was determined at room temperature, at least in duplicate, using an Ultrospec 2000 (Pharmacia, Cambridge, England) against a distilled water blank using a modification of the method of Guo et al. (1989). The turbidity of an unheated sample at a given pH was subtracted from the observed turbidity after heating, and the results were presented as an increase in turbidity due to heating.

Quantification of the pH 4.6 soluble amino group content in unheated and heat-treated samples at different pH values was performed in duplicate using the TNBS method of Adler-Nissen (1979) as modified by Panasiuk, Amarowicz, Kostyra, and Sijtsma (1998). Two millilitres of unheated or heat-treated NaCN (1 g

protein 100 ml^{-1}) was diluted with 6 ml of distilled water. The pH was adjusted to 4.6 using 0.01 N HCl and all samples were brought to a final volume of 10 ml. Following centrifugation (1620×*g*, 15 min), 0.125 ml of the supernatant was analysed for soluble amino group content. Absorbance at 340 nm was recorded using an Ultrospec 2000 (Pharmacia, Cambridge, England). The pH 4.6 soluble amino group content (mg l^{-1}) in unheated NaCN was subtracted from the amount in heated NaCN at each pH value. Distilled water (0.125 ml in place of test sample) was used as a blank. A standard curve was prepared with L-leucine.

2.4. Solubility analyses

Nitrogen solubility analysis was performed between pH 2.0 and 10.0 in duplicate on unmodified and cross-linked NaCN samples, as previously described (Flanagan & FitzGerald, 2002b).

2.5. Emulsifying analyses

Emulsifying analyses were performed in triplicate on unmodified and cross-linked NaCN samples according to the method of James and Patel (1988), with minor modifications (Flanagan & FitzGerald, 2002b). Emulsifying activity index (EAI) values were calculated by the corrected method of Cameron, Weber, Idziak, Neufeld, and Cooper (1991) where comparatively large values for EAI are indicative of smaller oil droplet sizes in protein stabilised emulsions.

2.6. Foaming analyses

Foam expansion (FE) and foam drainage stability (FS) analyses were performed on unmodified and cross-linked NaCN samples in duplicate according to the method of Slattery and FitzGerald (1998) with minor modifications (Flanagan & FitzGerald, 2002b).

2.7. Electrophoretic analyses

SDS-polyacrylamide gradient gel electrophoresis (SDS-PAGGE) was carried out on a Bio-Rad Protean II Xi electrophoretic system (Bio-Rad, Hertfordshire, England) in vertical-slab gels, with a 4% stacking gel and 9–15% gradient separating gel, using the method of Laemmli (1970). Urea PAGE was carried out by the method of Andrews (1983), with minor modifications (Flanagan & FitzGerald, 2002b).

3. Results and discussion

Polymers resulting from TGase-catalysed cross-linking of the caseins were evident by the appearance of bands at approximately 65 and above 94 kDa on SDS-PAGGE of the CL-a and CL-b samples (data not shown). These bands may be indicative of dimers and trimers of NaCN. However, some individual casein bands also remained in these cross-linked samples.

3.1. Heat stability of unmodified NaCN

The effect of heating at 140 °C on the turbidity and pH 4.6 soluble amino group content of unmodified and cross-linked NaCN is shown in Figs. 1–3. As the heating time at 140 °C of an unmodified NaCN solution (1 g 100 ml^{-1}) at different pH values was increased up to 20 min, turbidity also increased. However, after 60 min heating at 140 °C, reduced turbidity was observed for all samples, compared to the turbidity observed after 20 min heating. The increase in turbidity as heating time increased to 20 min may be attributed to casein aggregation, while the subsequent decrease in turbidity after heating for 60 min may be attributed to casein degradation. Guo et al. (1989) attributed an increase in the turbidity of NaCN solutions (1 g 100 ml^{-1}) at pH 7 heated from 120 to 140 °C for 60 min to aggregation, while decreased turbidity after heating at 145 and 150 °C was attributed to degradation of the caseins.

The rate and extent of turbidity increase was dependent on pH of the heated samples with rates of turbidity

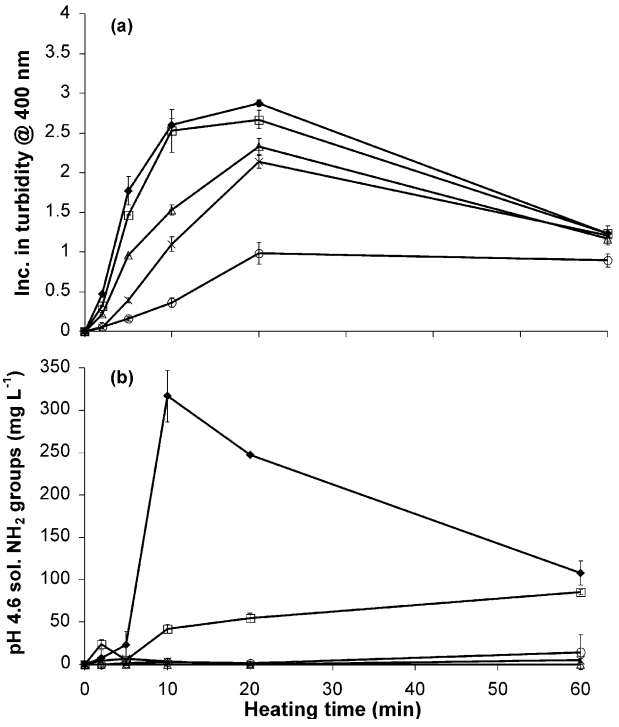

Fig. 1. Effect of heating unmodified sodium caseinate at 140 °C for different time intervals on (a) turbidity at 400 nm in 0.5 g protein 100 ml^{-1} and (b) pH 4.6 soluble amino group content at pH 6.0 (◆), 6.25 (□), 6.5 (△), 6.75 (∗) and 7.0 (○). Error bars show standard deviation.

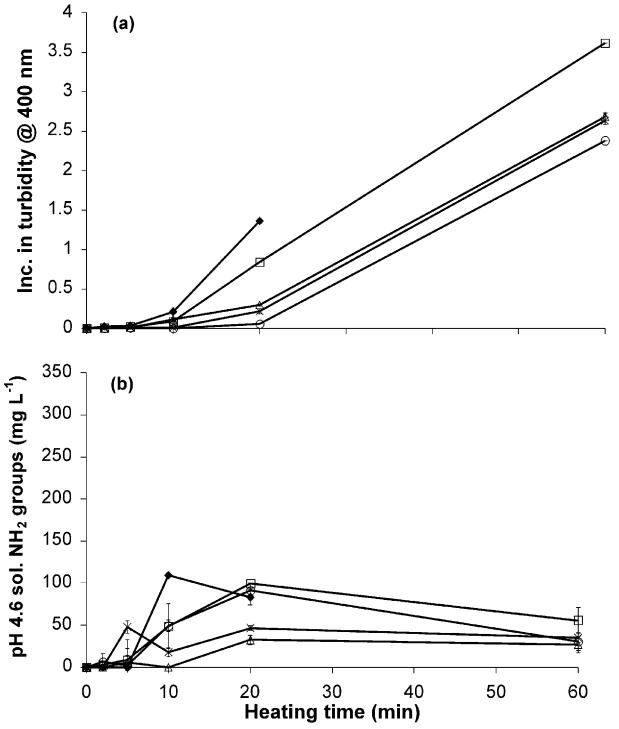

Fig. 2. Effect of heating transglutaminase-catalysed cross-linked sodium caseinate (CL-a; cross-linked for 185 min, [E:S] 1:50) at 140 °C for different time intervals on (a) turbidity at 400 nm in 0.5 g protein 100 ml^{-1} and (b) pH 4.6 soluble amino group content at pH 6.0 (◆), 6.25 (□), 6.5 (△), 6.75 (∗) and 7.0 (○). Error bars show standard deviation.

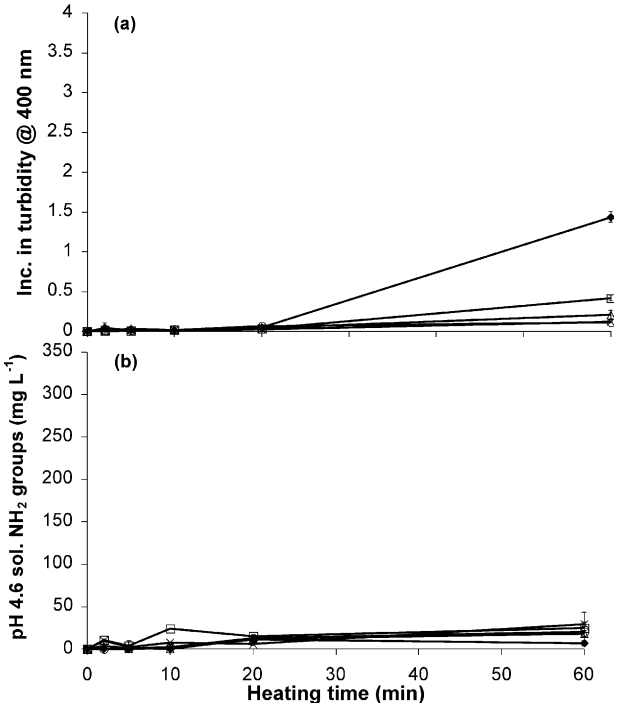

Fig. 3. Effect of heating transglutaminase-catalysed cross-linked sodium caseinate (CL-b; cross-linked for 185 min, [E:S] 1:20) at 140 °C for different time intervals on (a) turbidity at 400 nm in 0.5 g protein 100 ml^{-1} and (b) pH 4.6 soluble amino group content at pH 6.0 (◆), 6.25 (□), 6.5 (△), 6.75 (∗) and 7.0 (○). Error bars show standard deviation.

on heating up to 10 min as follows: pH 6.0 > 6.25 > 6.5 > 6.75 > 7.0 (Fig. 1a). Furthermore, overall turbidity increases on heating up to 20 min were in the order of pH 6.0 > 6.25 > 6.5 > 6.75 > 7.0, with the pH 6.0 sample displaying the highest increase in turbidity (2.90 absorbance units at 400 nm) after 20 min heating (Fig. 1a).

Urea PAGE patterns of samples heated at pH 6.0, 6.25 and 7.0 for 5, 10 and 60 min (Fig. 4, lanes 5–7, 9–11 and 13–15) revealed increased streaking of the heated samples compared to the unheated sample (Fig. 4, lanes 4, 8 and 12). Reduced intensities of bands eluting in the α- and β-casein regions were observed as heating time increased from 5 to 10 min (Fig. 4, lane 5–6, 9–10 and 13–14); this may also indicate that aggregation of the caseins had occurred. Furthermore, unmodified NaCN heated for 60 min did not display bands corresponding to material which did not enter the separating gel (Fig. 4, lanes 7, 11 and 15), in contrast to the samples heated for shorter times (Fig. 4, lanes 4–6, 8–10 and 12–14), indicative of heat-induced degradation. Similar to the results observed herein, little if any of the individual caseins remained after heating a NaCN solution at 140 °C and pH 7.0 for 60 min (Guo et al., 1989).

It also appears that the α-caseins are relatively more susceptible to heat-induced changes at 140 °C and pH

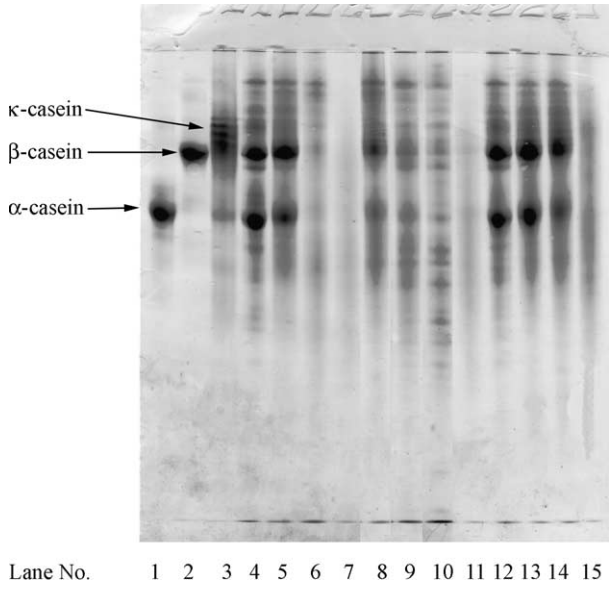

Fig. 4. Urea PAGE profiles of unmodified sodium caseinate (NaCN) heated at 140 °C. Lanes 1–3: α-, β- and κ-casein, respectively; lanes 4–7: NaCN, pH 6.0, heated for 0, 5, 10 and 60 min, respectively; lanes 8–12: NaCN, pH 6.25, heated for 0, 5, 10 and 60 min, respectively; lanes 12–15: NaCN, pH 7.0, heated for 0, 5, 10 and 60 min, respectively. Gels were loaded with 90 μg of the standards and 60 μg of the heat-treated samples.

6.0, as evidenced by the decrease in band intensity of the α-casein band compared to the β-casein band after 5 min heating (Fig. 4, lanes 4 and 5). It was initially reported that κ- and α_{s2}-caseins were more susceptible to thermally induced changes than α_{s1}- and β-casein as indicated by urea PAGE analysis (Guo et al., 1989). However, using N-terminal amino acid sequencing and mass spectrometry, Hustinx et al., (1997) subsequently reported that α_{s1}-casein is more susceptible to thermal degradation than the other caseins. More recently, Gaucheron, Mollé, Briard, and Léonil (1999) isolated peptides released from heating casein micelles at 120 °C for 30 min; 7 of the 12 identified peptides originated from α_{s1}-casein.

The increase in pH 4.6 soluble amino group content after 10 min heating may be due to heat-induced hydrolysis resulting in the liberation of peptides which are soluble at pH 4.6 (Fig. 1b). Deamidation of the caseins after heating under these conditions may also have occurred, rendering the deamidated caseins soluble at pH 4.6 and also increasing NPN content due to the formation of ammonia. Increases in the concentration of ammonia in heated NaCN solutions have previously been used to quantify rates of deamidation (Metwalli & von Boekel, 1998; van Boekel, 1999). The reason for the decrease in the pH 4.6 soluble amino group content on increasing heating time beyond 10 min at pH 6.0 is not clear. Very little change in the pH 4.6 soluble amino group concentration was evident on heating NaCN at pH 6.5, 6.75 and 7.0 up to 60 min (Fig. 1b). In contrast, almost 25% of the total nitrogen content was reported to be soluble at pH 4.6 on heating an aqueous NaCN solution (2.5 g 100 ml^{-1}) at 140 °C and pH 7.0 for 60 min (Hustinx et al., 1997).

The urea PAGE profile of the pH 6.25 sample (Fig. 4, lanes 8–11) differed from that of the pH 6.0 and 7.0 samples (Fig. 4, lanes 4–7, 12–15); the casein bands were less evident and after 20 min heating a large number of distinct new bands appeared indicating heat-induced cleavage of peptide bonds (Fig. 4, lane 10). However, these degradation products at pH 6.25 did not appear to be soluble at pH 4.6 as the pH 4.6 soluble amino group content was lower for the pH 6.25 than the pH 6.0 sample after 20 min heating (Fig. 1b).

Overall, heating at 140 °C had a major effect on the turbidity, pH 4.6 soluble amino group content and electrophoretic profiles of NaCN (Fig. 1). It appears that most of these changes may be attributed to heat-induced hydrolysis of the caseins, thereby altering the heat stability of NaCN. The pH at which the NaCN solution was heated also had a major effect on the heat stability, with heating at pH 6.0 resulting in relatively lower heat stability than heating at pH 7.0. In agreement with these findings, Hustinx et al. (1997) observed greater quantities of pH 4.6 soluble nitrogen liberated on heating NaCN at 140 °C for 60 min at pH 6.0 compared to the quantities liberated at pH 7.0.

3.2. Heat-treatment of CL-a sample

The effect of heating at 140 °C between pH 6.0 and 7.0 on the turbidity, and pH 4.6 soluble group content of TGase-catalysed cross-linked NaCN does not appear to have been previously reported. NaCN, which had been cross-linked with TGase for 185 min at an [E:S] of 1:50 (CL-a), displayed remarkably different turbidity profiles on heating at 140 °C at different pH values than unmodified NaCN (Fig. 2a). Increases in turbidity were only observed after 20 and 60 min heating, and a pH effect was again evident with turbidity at pH $6.0 > 6.25 > 6.5 > 6.75 > 7.0$. The pH 6.0 sample precipitated after 60 min heating at 140 °C; this prevented measurement of turbidity and quantification of soluble amino group content for this sample.

Higher turbidity was observed for the CL-a sample heated for 60 min compared to 20 min (Fig. 2a). However, the increased turbidity on 60 min heating may not necessarily be related to aggregation. While aggregated material appeared at the top of the separating gel for pH 6.0, 6.25 and 7.0 samples on heating for 0, 5 and 10 min (Fig. 5, lanes 4–6, 8–10 and 12–14), this material almost completely disappeared on heating for 60 min (Fig. 5, lanes 7, 11, 15). The disappearance of individual

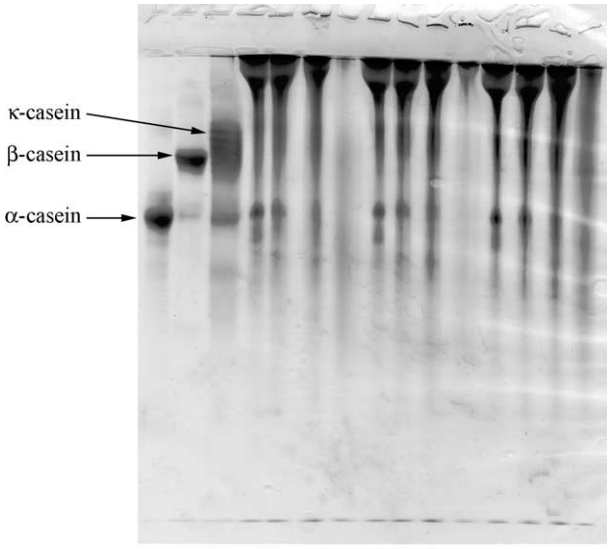

Fig. 5. Urea PAGE profiles of sodium caseinate cross-linked with transglutaminase (CL-a; cross-linked for 185 min, [E:S] 1:50) and heated at 140 °C. Lanes 1–3: α-, β- and κ-casein, respectively; lanes 4–7: CL-a, pH 6.0, heated for 0, 5, 10 and 60 min, respectively; lanes 8–11: CL-a, pH 6.25, heated for 0, 5, 10 and 60 min, respectively; lanes 12–15: CL-a, pH 7.0, heated for 0, 5, 10 and 60 min, respectively. Gels were loaded with 90 μg for the standards and 60 μg for the heat-treated samples.

casein bands is not particularly evident until after 10 min heating (Fig. 5, lanes 6, 10 and 14).

In the region of 40–100 mg l^{-1} of pH 4.6 soluble amino groups were observed for all CL-a samples over the pH range studied (Fig. 2b). There was a notable reduction in the pH 4.6 soluble amino group content of the CL-a sample heated for 10 min at pH 6.0 (110 mg l^{-1}) compared to unmodified sodium caseinate (317 mg l^{-1}), at the same pH. However, the CL-a samples displayed increased susceptibility to formation of pH 4.6 soluble amino groups between pH 6.5 and 7.0 compared to the concentration in unmodified NaCN at these pH values (Figs. 1b and 2b).

3.3. Heat-treatment of cross-linked sample CL-b

With the exception of the CL-b sample which showed a small increase in turbidity after 60 min heating at pH 6.0, increasing heating time from 0 to 60 min at 140 °C resulted in very little change in turbidity in the CL-b cross-linked NaCN (Fig. 3a). Urea PAGE was not performed on the CL-b samples as preliminary investigations of the unheated CL-b sample showed that protein bands corresponding to individual caseins were essentially absent. Very low quantities of pH 4.6 soluble material (<30 mg l^{-1}) were evident in the CL-b sample on heating at 140 °C over 60 min at all pH values examined (Fig. 3b). This was in contrast to the quantity of pH 4.6 soluble material observed on heating the unmodified (up to 316 mg l^{-1}) and the CL-a samples (between 0 and 110 mg l^{-1}; Figs. 1b and 2b).

Overall, cross-linking NaCN with TGase resulted in lower turbidity and pH 4.6 soluble amino group content on heating at 140 °C over shorter heating times (2–20 min), compared to heating unmodified sodium caseinate. This indicated that the cross-linked products were more heat stable than unmodified NaCN at the pH values studied. After 60 min heating however, the electrophoretic and turbidity profiles indicate that some heat-induced changes in the cross-linked products had occurred.

The much improved heat stability properties of NaCN between pH 6.0 and 7.0 following incubation with TGase does not appear to have been previously reported. Reduced (1.5%) heat stability was observed on heating a TGase cross-linked NaCN solution (3 g 100 ml^{-1}) at pH 6.8 for 20–30 s at 120 °C, as expressed by the percentage protein present in the supernatant after centrifugation (4500×g, 15 min) relative to the initial protein content (Lorenzen, 2000). O'Sullivan et al. (2001) treated skim milk with TGase (16 h, 6 °C, [E:S] 1:2000) prior to freeze or spray drying. The reconstituted skimmed milk (9.0% total solids) exhibited markedly increased heat stability in the pH range of minimum stability of the non-cross-linked control, i.e. pH 6.8–7.1. TGase cross-linking was also shown to increase the heat stability of reconstituted concentrated skim milk at pH values greater than 6.7, compared to control (O'Sullivan et al., 2001).

Resistance to coagulation, gelation and/or sedimentation is a prerequisite for the application of NaCN in formulated food products which may undergo severe heat treatment, e.g. UHT processing. The improvements in the heat stability properties of NaCN on modification with TGase reported herein indicate that the cross-linked products may find numerous potential commercial applications.

3.4. Solubility

Both the CL-a and the CL-b samples displayed very similar solubility profiles between pH 2.0 and 10.0 (Fig. 6). Improved solubility was observed for both cross-linked samples at pH 2.0 and 3.0, where almost 100% solubility was observed compared to 57 and 74% solubility for unmodified NaCN at these pH values, respectively. At pH 5.0, solubility values of 34 and 40% were observed for the CL-a and CL-b samples, respectively, which were greater than the control (5% solubility; Fig. 6).

The products of minimal cross-linking (3 min, [E:S] 1:100) were previously shown to display decreased solubility at pH 2.0 and 3.0 compared to unmodified NaCN (Flanagan & FitzGerald, 2002a). However, limited (42 min, [E:S] 1:100) and extensive (290 min, [E:S] 1:10) TGase treatment resulted in almost 100% solubility at these pH values, compared to 73 and 58% solubility for unmodified NaCN at pH 2.0 and 3.0, respectively. At pH 5.0, the solubilities were 5, 45, 3 and 91% for unmodified NaCN, and the minimal, limited and extensively cross-linked samples, respectively (Flanagan & FitzGerald, 2002a).

Improved nitrogen solubility of α_{s1}-casein at pH 5 and 6 compared to intact substrate on incubation with TGase isolated from guinea pig liver was reported (Motoki et al., 1984). Increased solubility at pH 5 and 5.5 compared to unmodified α_{s1}-casein was observed

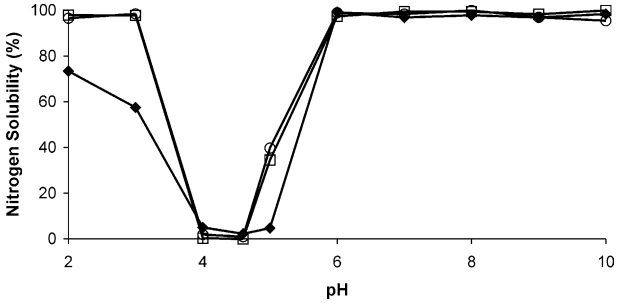

Fig. 6. Effect of pH on the nitrogen solubility (%) of unmodified sodium caseinate (NaCN, ◆), NaCN cross-linked with transglutaminase for 185 min, [E:S] 1:50; (CL-a □) and NaCN cross-linked with transglutaminase for 185 min, [E:S] 1:20; (CL-b ○).

following deamidation of citraconylated α_{s1}-casein using soluble guinea pig liver (Motoki et al., 1986) and immobilised microbial TGases (Nonaka et al., 1996). No change in the nitrogen solubility of microbial TGase-treated NaCN was observed at pH 7 (Lorenzen, 2000). However, NaCN generally displays 100% solubility at pH 7.0 (Flanagan & FitzGerald, 2002a).

3.5. Emulsifying properties

The EAI of unmodified NaCN, the CL-a and CL-b samples are summarised in Table 1. Improved EAI was observed for the CL-a sample at pH 5.0 compared to unmodified NaCN. However, the CL-a and CL-b samples exhibited decreased EAI between pH 2.0 and 4.6 compared to unmodified NaCN. The CL-b sample displayed improved EAI at pH 10.0 compared to unmodified NaCN (Table 1).

The CL-a sample exhibited improved ES at pH 4.6, 5.0 and 10.0, while the CL-b sample displayed improved ES at pH 4.6 and between pH 7.0 and 10.0 (Table 1). However, the EAI of both CL-a and CL-b were low at pH 4.6.

Similar results were observed on incubating NaCN with TGase to minimal, limited and extensive extents of cross-linking (Flanagan & FitzGerald, in press). Improved EAI was observed at pH 5.0, 9.0 and 10.0 for all extents of cross-linking, while improved ES was observed at pH 5.0 on extensive cross-linking, compared to unmodified NaCN. Improved ES of TGase-treated NaCN was reported at an unspecified pH value; however, the cross-linked NaCN displayed lower oil binding capacity than non-cross-linked control (Lorenzen, 2000).

While improvements in the emulsifying properties of NaCN and individual caseins have been reported by rheological determinations at specific pH values (Dickinson & Yamamoto, 1996; Dickinson et al., 1999; Faergemand & Murray, 1998; Faergemand, Otte, & Qvist, 1998; Liu & Damodaran, 1999), little information appears in the literature on the effect of pH on TGase-treated NaCN stabilised emulsions.

3.6. Foaming properties

Apart from pH 2.0, where the control and the CL-a and CL-b samples displayed similar FE values (in the region of 800–900%), the products of cross-linking displayed FE values of less than 500% (Table 1). In general, the FS of the cross-linked products were much reduced, with the exception at pH 4.6, where the CL-a and CL-b samples displayed 19 and 10% FS, compared to 1% FS for unmodified NaCN (Table 1). As the CL-a and CL-b samples displayed similar solubility values to those of unmodified NaCN at pH 4.6 (Table 1), the increases in FS may be attributed to the introduction of covalent ε-(γ-glutamyl)lysine isopeptide bonds.

However, reduced extents of TGase modification than those studied herein have previously been shown to improve FE at pH 2.0, 3.0, 9.0 and 10.0, compared to unmodified NaCN, although extensive cross-linking resulted in very poor foamability (Flanagan & FitzGerald, in press). Reduced foam volume and foam stability of TGase-catalysed cross-linked NaCN compared to non-cross-linked NaCN were reported (Lorenzen, 2000).

It appears that incubation of NaCN with TGase alone is of limited benefit for improvement in the emulsifying and foaming properties of NaCN. However, it has previously been shown that combining TGase treatment and hydrolysis with a *Bacillus* proteinase (Protamex), resulted in significantly improved emulsifying and foaming properties compared to unmodified NaCN or the products generated by hydrolysis or cross-linking with TGase per se (Flanagan & FitzGerald, in press).

Table 1
Effect of pH on the emulsifying activity index (EAI), emulsion stability (ES), foam expansion (FE) and foam stability (FS) of unmodified sodium caseinate (NaCN), and NaCN cross-linked with transglutaminase for 185 min at an [E:S] of 1:50 (CL-a) and 1:20 (CL-b). Values presented are means±standard deviation

pH	EAI ($m^2\ g^{-1}$)			ES (%)			FE (%)			FS (%)		
	NaCN	CL-a	CL-b	NaCn	CL-a	CL-b	NaCN	CL-a	CL-b	NaCN	CL-a	CL-b
2.0	24±2.3	34±1.4	19±1.8	89±6.2	90±10.1	100±6.4	832±19	881±1	834±10	59±3.2	26±3.2	14±2.4
4.0	22±0.1	4±0.6	4±0.1	55±3.1	64±8.7	44±0.8	670±10	–[a]	–	49±0.1	23±1.7	11±0.9
4.6	8±1.9	4±0.2	3±0.3	11±1.5	70±9.8	88±10.3	–	–	–	0	19±1.5	10±0.5
5.0	9±0.9	18±0.3	10±0.8	12±5.1	41±9.3	22±3.4	–	–	–	31±1.1	29±2.1	18±3.1
6.0	35±0.7	23±0.2	29±1.2	71±2.0	72±11.2	80±3.3	823±50	–	–	41±7.4	0	0
7.0	40±0.8	37±1.5	26±1.3	57±1.6	60±8.2	89±5.9	917±70	–	–	44±0.2	0	0
8.0	41±1.7	43±3.4	45±0.2	52±5.3	61±2.0	77±5.0	934±59	–	–	35±5.0	0	0
10.0	43±2.2	41±1.5	56±1.2	58±3.3	74±0.6	78±4.4	750±1	–	–	24±1.6	0	0

[a] –, < 500% FE.

4. Conclusions

The heat stability properties of TGase cross-linked NaCN between pH 6.0 and 7.0 do not appear to have been previously characterised. NaCN which was cross-linked with TGase to two different extents displayed improved heat stability compared to unmodified NaCN as indicated by lower turbidity and pH 4.6 soluble amino group content observed on heating at 140 °C. Both unmodified and cross-linked NaCN were found to be more heat stable on heating at pH 7.0 compared to pH 6.0. The results indicate that TGase may be used to modify the heat stability properties of NaCN. Therefore, limitations to the utilization of NaCN in products which undergo severe heat treatment may potentially be overcome by TGase cross-linking.

References

Adler-Nissen, J. (1979). Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *Journal of Agricultural and Food Chemistry*, *27*, 1256–1262.

Andrews, A. T. (1983). Proteinase in normal bovine milk and their action on caseins. *Journal of Dairy Research*, *50*, 45–55.

Cameron, D. R., Weber, M. E., Idziak, E. S., Neufeld, R. J., & Cooper, D. G. (1991). Determination of interfacial areas in emulsions using turbidimetric and droplet size data: correction of the formula for emulsifying activity index. *Journal of Agricultural and Food Chemistry*, *39*, 655–659.

Dickinson, E., Ritzoulis, C., Yamamoto, Y., & Logan, H. (1999). Ostwald ripening of protein-stabilized emulsions: effect of transglutaminase crosslinking. *Colloids and Surfaces B: Biointerfaces*, *12*, 139–146.

Dickinson, E., & Yamamoto, Y. (1996). Rheology of milk protein gels and protein-stabilized emulsion gels cross-linked with transglutaminase. *Journal of Agricultural and Food Chemistry*, *44*, 1371–1377.

Faergemand, M., & Murray, B. S. (1998). Interfacial dilational properties of milk proteins cross-linked by transglutaminase. *Journal of Agricultural and Food Chemistry*, *46*, 884–890.

Faergemand, M., Otte, J., & Qvist, K. B. (1998). Emulsifying properties of milk proteins cross-linked with microbial transglutaminase. *International Dairy Journal*, *8*, 715–723.

Flanagan, J., & FitzGerald, R. J. (2002a). Physicochemical and nitrogen solubility properties of *Bacillus* proteinase hydrolysates of sodium caseinate incubated with transglutaminase pre- and post-hydrolysis. *Journal of Agricultural and Food Chemistry*, *50*, 5429–5436.

Flanagan, J., & FitzGerald, R. J. (2002b). Functionality of *Bacillus* proteinase hydrolysates of sodium caseinate. *International Dairy Journal*, *12*, 737–748.

Flanagan, J., & FitzGerald, R. J. (in press). Functional properties of *Bacillus* proteinase hydrolysates of sodium caseinate incubated with transglutaminase pre- and post-hydrolysis. *International Dairy Journal*.

Gaucheron, F., Mollé, D., Briard, V., & Léonil, J. (1999). Identification of low molar mass peptides released during sterilization of milk. *International Dairy Journal*, *9*, 515–521.

Guo, M. R., Fox, P. F., Flynn, A., & Kindstedt, P. S. (1996). Heat-induced modifications of the functional properties of sodium caseinate. *International Dairy Journal*, *6*, 473–483.

Guo, M., Fox, P. F., Flynn, A., & Mohammad, K. S. (1989). Heat-induced changes in sodium caseinate. *Journal of Dairy Research*, *56*, 503–512.

Hustinx, J. C. A., Singh, T. K., & Fox, P. F. (1997). Heat-induced hydrolysis of sodium caseinate. *International Dairy Journal*, *7*, 207–212.

Jahaniaval, F., Kakuda, Y., Abraham, V., & Marcone, M. F. (2000). Soluble protein fractions from pH and heat treated sodium caseinate: physicochemical and functional properties. *Food Research International*, *33*, 637–647.

James, M. J., & Patel, P. D. (1988). *Development of a standard oil-in-water emulsification test for proteins*. Research Report Number 631, British Food Manufacturing Industries Research Association.

Kuraishi, C., Yamazaki, K., & Susa, Y. (2001). Transglutaminase: its utilization in the food industry. *Food Reviews International*, *17*, 221–246.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of a bacteriophage T4. *Nature*, *227*, 680–686.

Liu, M., & Damodaran, S. (1999). Effect of transglutaminase-catalysed polymerisation of β–casein on its emulsifying properties. *Journal of Agricultural and Food Chemistry*, *47*, 1514–1519.

Lorenzen, P. C. (2000). Techno-functional properties of transglutaminase-treated milk proteins. *Milchwissenschaft*, *55*, 667–670.

Lorenzen, P. C., & Schlimme, E. (1998). Properties and potential fields of application of transglutaminase preparations in dairying. *Bulletin of the IDF*, *332*, 47–53.

Metwalli, A. A. M., & van Boekel, M. A. J. S. (1998). On the kinetics of heat-induced deamidation and breakdown of caseinate. *Food Chemistry*, *61*, 53–61.

Motoki, M., Nio, N., & Takinami, K. (1984). Functional properties of food proteins polymerized by transglutaminase. *Agricultural and Biological Chemistry*, *48*, 1257–1261.

Motoki, M., Seguro, K., Nio, N., & Takinami, K. (1986). Glutamine-specific deamidation of α_{s1}-casein by transglutaminase. *Agricultural and Biological Chemistry*, *50*, 3025–3030.

Motoki, M., & Seguro, K. (1998). Transglutaminase and its use for food processing. *Trends in Food Science and Technology*, *9*, 204–210.

Nielson, P. M. (1995). Reactions and potential industrial applications of transglutaminase. Review of literature and patents. *Food Biotechnology*, *9*, 119–156.

Nonaka, M., Sawa, A., Matsuura, Y., Motoki, M., & Nio, N. (1996). Deamidation of several food proteins using free and immobilized Ca^{2+} -independent microbial transglutaminase. *Bioscience Biotechnology and Biochemistry*, *60*, 532–533.

O'Connell, J. E., & Fox, P. F. (2002). Heat stability of milk and heat induced changes. In P. F. Fox, & P. L. H. McSweeney (Eds.), *Advanced Dairy Chemistry I. Proteins,* (3rd ed.). New York: Kluwer Academic Press.

O'Sullivan, M. M., Lorenzen, P. C., O'Connell, J. E., Kelly, A. L., Schlimme, E., & Fox, P. F. (2001). Influence of transglutaminase on the heat stability of milk. *Journal of Dairy Science*, *84*, 1331–1334.

Panasiuk, R., Amarowicz, R., Kostyra, H., & Sijtsma, L. (1998). Determination of α-amino nitrogen in pea protein hydrolysates: a comparison of three analytical methods. *Food Chemistry*, *62*, 363–367.

Schorsch, C., Carrie, H., & Norton, I. T. (2000). Cross-linking micelles by a microbial transglutaminase: influence of cross-links in acid-induced gelation. *International Dairy Journal*, *10*, 529–539.

Singh, H., & Creamer, L. K. (1992). Heat stability of milk. In P. F. Fox (Ed.), *Advanced Dairy Chemistry I. Proteins* (pp. 621–656). London: Elsevier Applied Science.

Slattery, H., & FitzGerald, R. J. (1998). Functional properties and bitterness of sodium caseinate hydrolysates prepared with a Bacillus proteinase. *Journal of Food Science*, *63*, 418–422.

van Boekel, M. A. J. S. (1999). Heat-induced deamidation, phosphorylation and breakdown of caseinate. *International Dairy Journal*, *9*, 237–241.

Zhu, Y., Rinzema, A., Tramper, J., & Bol, J. (1995). Microbial Transglutaminase—a review of its production and application in food processing. *Applied Microbiological Biotechnology*, *44*, 277–282.