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Protein interactions in comminuted meat gels containing emulsified corn oil

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Abstract

Model comminuted meat gels incorporating corn oil droplets, emulsified either with salt-soluble meat proteins (SSMP) or with soy protein isolate (SPI), were prepared by heat treatment of meat paste at 90 °C, and their strength was evaluated by applying a uniaxial compression test. The presence of emulsified oil droplets resulted in a significant reduction of the gel network strength, the extent of decrease being independent of the type of protein emulsifier used. Gels prepared with SSMP, on the other hand, suffered a less pronounced structure disruption by oil droplet incorporation and the extent of disruption depended on the type of protein used for oil emulsification. Competitive adsorption studies in emulsions between SSMP and SPI indicated that the former may partly displace the latter from oil droplet surface, thus, offering binding sites to which the SSMP molecules of the gel network may become attached during the development of system structure.

Keywords: Meat gels; Soy protein isolate; Gelation; Fracture properties

1. Introduction

Finely comminuted meat batters of products, such as frankfurters or bologna are homogeneous mixtures consisting mainly of disrupted muscle protein fibres, fat particles, salt and water, where a number of meat salt-soluble proteins, such as myosin, actin and actomyosin are found, together with proteins added in the form of isolates, e.g. soy protein isolate (Barbut, 1994). Soy protein is incorporated in these products in a prehydrated form, at levels up to 4%, to improve product quality through its moisture and fat binding ability as well, as through its emulsifying and structure-forming potential (Rhee, 1994). Other proteins of plant origin and animal proteins, such as milk proteins, may also find use in these systems. Lupin protein isolate, for example, was found to enhance product functionality, both in terms of liquid retention, upon cooking, as well as with respect to structure development (Alamanou, Doxastakis, Bloukas, & Paneras, 1996; Mavrakis, Doxastekis, & Kiosseoglou, 2003).

Although, incorporation of soy or other proteins may contribute towards the improvement of comminuted meat product properties, product preparation and behaviour during processing and cooking depend to a great extent on the remarkable functionality of meat salt-soluble proteins which, following myofibril dissociation during ingredient mixing in the presence of salt, are extracted to some extent and in this form adsorb at the fat particle surfaces, acting as emulsifiers and emulsion stabilizers. Proteins coagulate during thermal processing and/or cooking, resulting in the formation of a gel-like structure, by binding together the batter structural units, e.g. the muscle myofibrils and the emulsified fat particles and contributing to the development of the final product texture (Acton & Dick, 1989; Barbut, 1995; Xiong, 1997).

A problem, however, associated with comminuted meat product consumption is their high animal fat content which may reach concentrations as high as 50% and there has been a trend in recent years to develop products that are either low in fat or that have animal

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fat replaced by vegetable oil (Colmenero, 1996, 2000). The second approach has the advantage that product texture is not adversely affected, as is the case in the low-fat products, provided the oil is incorporated in the form of stable oil droplets which do not coalesce, during product processing or cooking, a process that would result in liquid loss and poor quality.

Soy proteins are well known for their emulsifying and emulsion-stabilizing properties. Although these functional properties are considered important in comminuted meat products, the exact role of soy protein molecules in the presence of salt-soluble meat proteins (SSMP) has not yet been clearly established. The aim of this study was to investigate the suitability of soy protein isolate (SPI) as an emulsion-stabilizer in model comminuted meat products, where vegetable oil droplets, emulsified with the isolate, are incorporated in the place of animal fat particles. An additional target was to investigate the mechanism of soy protein functionality in the presence of SSMP and the way they contribute to structure formation of the emulsion-gel system resulting upon heating of the initial raw batter mixture.

2. Materials and methods

2.1. Materials

Comminuted lean pork, ground through a 4.5 mm plate, was obtained from a local meat market. It was then packed in moisture-proof bags and frozen at −20 °C. SPI was prepared from soybeans kindly provided by the Cotton Institute, Thessaloniki. The beans were comminuted into flour, the oil was extracted with petroleum ether and the resulting defatted flour was mixed with water (1:10 w/w), followed by protein extraction at pH 8.5 under continuous stirring. The protein of the resulting extract was precipitated at pH 4.6 and, following washing with distilled water of the precipitate, its pH was adjusted to 7 and freeze-dried. The protein content of the resulting soy protein isolate was 91% (on a dry-weight basis) as determined by the Kjeldahl method (N% × 6.25).

Refined corn oil was obtained from the local market and was used without any further treatment.

2.2. Preparation of salt-soluble proteins

Following thawing, 100 g of comminuted meat were treated with 1 l 0.8 M NaCl solution at 4-5 °C. The pH of the mixture was continuously adjusted to 6.5, while mixing, with the aid of a mechanical stirrer for 1 h. The protein extract was separated from the slurry by centrifugation at 3000*g* for 1 h and, following removal of NaCl by dialysis for 24 h, the SSMP were obtained in their dry form by applying freeze-drying to the extract.

2.3. Emulsion preparation

Oil in water (o/w) emulsions (30% in oil) were prepared by first dissolving SPI, SSMP or their mixtures in a phosphate buffer (Na_2HPO_4, KH_2PO_4, 0.6 M NaCl) at pH 6.5, followed by dropwise addition of corn oil to the continuous phase (1% in protein), while mixing with the aid of a mechanical stirrer. The resulting crude emulsion was then homogenized at 9500 rpm for 1 min with the use of an Ultra Turrax T25 homogenizer (IKA Instruments), equipped with a S 25KG-25F dispersing tool.

The oil droplet size distribution of the resulting emulsions was determined by employing a Malvern Mastersizer 2000 (Malvern Instruments).

2.4. Protein adsorption studies

The amount of protein adsorbed per unit interface of emulsion was determined as follows: Following centrifugation of 100 ml of each emulsion at 1000*g*, after the addition of an equal quantity of the phosphate buffer of pH 6.5, the cream was recovered and 30 ml of a Tris buffer of pH 6.8, containing 1% SDS, was added. The mixture was agitated and stored at −20 °C for 24 h. Following thawing and centrifugation, which resulted in emulsion breakdown and oil separation, the water phase, containing the adsorbed protein, was recovered and analysed for protein content by the Lowry method (Lowry, 1951).

The amount of protein adsorbed per unit interface, Γ_s, was calculated using the equation,

$$\Gamma_s(\mathrm{mg/m^2}) = \Gamma_T/S_T, \quad (1)$$

where Γ_T is the amount of protein adsorbed and S_T is the total emulsion interface derived by the equation,

$$S_T = V6/d_{3,2}, \quad (2)$$

where V is the oil volume and $d_{3,2}$ the mean surface diameter.

The composition of the recovered adsorbed protein was determined by SDS–PAGE analysis in the presence of mercaptoethanol, as described by Laemli (1970), using an Apelex Model ST 1006T vertical electrophoresis apparatus (SciePlus, France). The stacking and the resolving gels contained 3% and 10% acrylamide, respectively.

2.5. Comminuted meat and SSMP gel preparation

To prepare model comminuted meat gels, 200 g of partially thawed comminuted lean meat was mixed with 100 g ice and 7 g NaCl, using a blender fitted with knives, operating at low speed. The resulting paste was left one night at −4 °C to effect extraction of the salt-soluble proteins. Cylindrical cells of 1 cm diameter, made from aluminium foil and reinforced with multiple layers of

plastic tape, were then filled with the meat paste and the ends were sealed very carefully to prevent leakage, before immersing in a 90 °C water bath for 1 h. Following storage at room temperature for 24 h, the cells were cut open to obtain cylindrical samples 1 cm in height.

In order to prepare comminuted meat gels containing emulsified oil droplets, a suitable quantity of emulsion cream, recovered by centrifugation of freshly prepared emulsions with SSMP or SPI, was added to the meat paste and thoroughly mixed to obtain a paste incorporating 30% corn oil. The paste was then treated, as described above, to obtain cylindrical gel samples.

SSMP gels (15% in protein) were prepared by dispersing the dried meat proteins in a phosphate buffer of pH 6.5, under continuous stirring with the aid of a magnetic stirrer, followed by filling into cells and heating at 90 °C for 1 h. To prepare SSMP gels incorporating oil droplets (30%), an emulsion stabilized by SSMP or SPI (1% in protein) was first prepared, as described above, and a suitable quantity of SSMP was added and thoroughly dispersed, with the aid of a magnetic stirrer, in order to obtain emulsions containing 15% protein in their continuous phase. The emulsions were then filled into cylindrical cells and gelled at 90 °C for 1 h.

2.6. Measurement of gel fracture properties

The cylindrical gel samples were subjected to 80% compression at a speed of 0.5 mm/s using the Stable Micro System TA-XT2i Texture Analyser, equipped with a 2.5-cm diameter compression cylinder. Vegetable oil was applied to the plates to act as lubricant, in order to avoid sample bulging. The resulting compression force-time curves were converted into stress–Hencky strain curves as follows (van Vliet, 1999):

The stress $G(t)$ was calculated by

$$G(t) = \frac{F_t}{A_t}, \tag{3}$$

where F_t is the force at time t and A_t is the corresponding surface area of the test sample given by

$$A(t) = \frac{L_o}{L_t} A_o, \tag{4}$$

where L_o and L_t are the original height and the height after time t, respectively.

The Hencky strain, E_H, was calculated from

$$E_H = \ln \frac{L_t}{L_o} \tag{5}$$

2.7. Measurement of liquid loss during cooking or upon compression

Cooking liquid loss of gels during preparation was determined by weighing the liquid lost when the gels were cut open. The ability of gels to retain liquid under pressure was determined according to the modified procedure of Funami, Yada, and Nakao (1998) from the liquid expressed from a 2-mm thick cylindrical gel sample compressed (10%) for 30 min between double layers of filter paper employing the Texture Analyser. The apparent expressible water percentage (AEW%) was calculated from the equation

$$\text{AEW \%} = \frac{(\text{weight before compression}) - (\text{weight after compression})}{(\text{weight before compression})} \times 100 \tag{6}$$

2.8. Statistical analysis

All the experiments were conducted at least three times and the data were analysed using a one-way ANOVA program. The level of significance was 95%. The LSD procedure was applied to identify significant differences between means.

3. Results and discussion

The effect of emulsified corn oil incorporation on the compressive behaviour of a model comminuted meat gel system is shown in Fig. 1. The presence of oil droplets, emulsified either with SSMP or SPI, resulted in a marked decrease of stress with strain values of the gel. Analysis of the curves indicated that the fracture stress and fracture strain parameter values of the comminuted meat gel decreased significantly, following oil droplet incorporation. However, no significant differences were observed between the two protein emulsifiers studied (Table 1). The decrease in the fracture properties of the gels, as a result of oil addition, was expected since the presence of fat contributes towards toughness reduction of processed/cooked products. This may be explained by

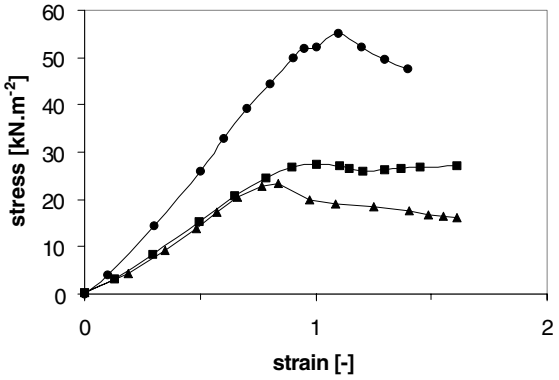

Fig. 1. Stress–strain curves for comminuted meat gels obtained by heating (at 90 °C for 1 h) meat paste without (●) or incorporating 30% emulsified oil with SSMP (■) or SPI (▲).

Table 1
Fracture stress and strain parameter values of comminuted meat (CMG) and SSMP gels (SSMPG) containing 30% oil emulsified with SSMP or SPI

Sample	$d_{3,2}$ (μm)	Fracture stress (kN/m^2)	Fracture strain (–)
Group 1			
CMG	–	56[a]	1.07[a]
CMG + SSMP emulsion	9.5[a]	28[b]	0.89[b]
CMG + SPI emulsion	5.8[b]	24[b]	0.88[b]
Group 2			
SSMPG	–	16.1[a]	0.52[a]
SSMPG + SSMP emulsion	10.0[a]	13.8[b]	0.60[a]
SSMPG + SPI emulsion	5.7[b]	9.4[c]	0.52[a]
Group 3			
SSMPG + SPI emulsion	5.8[a]	9.4[a]	0.52[a]
SSMPG + den.SPI emulsion	5.7[a]	7.6[b]	0.55[a]

[a,b]Different superscripts within each group of samples indicate significant differences at $P < 0.05$.

the fact that when fat particles or oil droplets of relatively large size (>1 μm) are incorporated into protein gel systems, they bring about a reduction in gel strength because of gel protein network disruption, irrespective of whether their surfaces, as a result of adsorbed protein molecules, interact with the gel matrix or not (Aguilera, 1992).

The extent of mechanical strength reduction of a gel system, upon incorporation of oil droplets, should depend on the droplet size, with the reduction being more pronounced in the case of the larger sizes, and also on the intensity of gel matrix-droplet surface interactions (Koidis, Paraskevopoulou, & Kiosseoglou, 2002). During heat treatment, the emulsion droplet size may increase due to coalescence between the oil droplets. This may lead to a further decrease in gel strength if the droplets are introduced into a gel-forming system. In order to investigate the possibility of oil droplet coalescence during heat treatment, emulsions were prepared with SSMP or SPI and heated at 95 °C for 1 h, followed by dispersion in a phosphate buffer of pH 6.5 containing 1% SDS and 0.2% mercaptoethanol under continuous agitation for 2 h with the help of a magnetic stirrer, at 50 °C. Preliminary experiments indicated that such treatment was necessary to break up any flocs formed during heating at 95 °C. It appeared that no significant increase in emulsion droplet size took place, suggesting that this may also have been the case in comminuted meat gel preparation containing emulsified SSMP or SPI. The absence of droplet coalescence may also partly explain the relatively low liquid loss during the gel preparation process and also during the compression of the thin sample discs in the texture analyser (Fig. 2).

Gels prepared with SSMP exhibited a much lower gel strength than those of the meat paste (Fig. 3). Incorporation of emulsified oil droplets into these systems also resulted in a decrease of their fracture stress values,

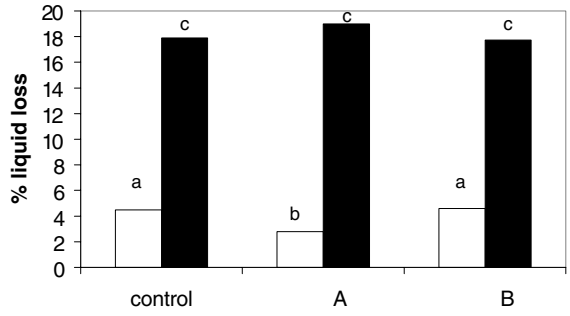

Fig. 2. Liquid loss during preparation (□) or upon compression (■) of comminuted meat gels containing 30% emulsified oil with SSMP (A) or SPI (B). [a–c]Different superscripts indicate significant differences at $P < 0.05$.

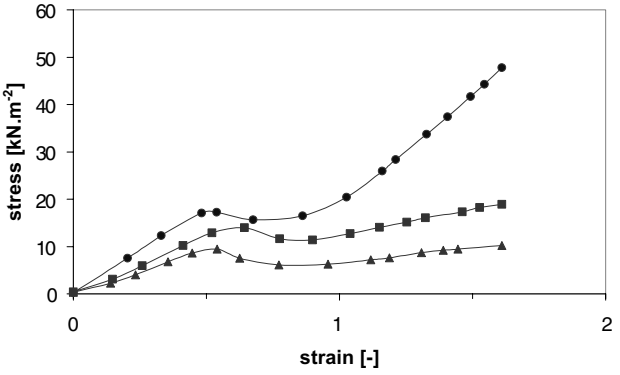

Fig. 3. Stress–strain curves for SSMP gels obtained by heating a 15% SSMP solution at 90 °C for 1 h without (●) or incorporating 30% emulsified oil with SSMP (■) or SPI (▲).

the reduction being more pronounced in the case of SPI emulsified oil droplet incorporation (Table 1). The water phase of a comminuted meat emulsion batter is a complex mixture of muscle fibres, myofibrils and myosin, actin, actomyosin and other myofibrillar as well as sarcoplasmic and stromal proteins, occurring either in the dissolved state or in the form of aggregates (Bailey, 1989). Following processing at 60 to 70 °C or cooking at higher temperatures, protein denaturation, followed by protein–protein interaction and gel network structure formation, takes place with myosin molecules playing a dominant role in network structure development. The myosin molecules, under relatively moderate heating conditions, initially form aggregates through interactions involving their globular heads, followed by rod unfolding at higher temperatures, which leads to myosin aggregate interaction and gel network formation (Barbut, 1994). Comminuted meat products, therefore, can be treated as multicomponent gels with a primary matrix dominated by meat proteins, especially myosin (Foegeding & Hamann, 1992). To explain, however, the higher compressive strength of the comminuted meat gel as compared to that of the SSMP one, the important gel structure-strengthening role of the meat fibres and

myofibrils present in the former system, has to be taken into account. As Aguilera (1992) has pointed out, fibre incorporation in a protein gel may lead to a stronger gel structure, on condition that interactions take place between the protein network and the fibres. In comminuted meat batters, a fraction of proteins should be extracted, depending mainly on pH and NaCl content, as well as on the degree of myofibril disruption and the meat origin (Xiong, 1997). During heat treatment, the extracted meat protein molecules denature and interact with each other and with the proteins left behind in the fibres and the myofibrils, leading to the development of a fibre-reinforced SSMP gel system.

The domination of the SSMP gel structure by the muscle fibres and myofibrils and the dramatic increase of the network strength as a result of their presence, is probably responsible for the non-detection of differences, as far as resistance to fracture is concerned, between the comminuted meat gel systems containing oil droplets emulsified either with SSMP or SPI. Incorporation of oil droplets in the SSMP gel system, however, resulted in networks exhibiting a different compressive strength, depending on the type of protein employed as oil emulsifier. This indicates that the adsorbed (on oil droplet surface) soy protein molecules interacted to a lesser extent with the gel network and resulted in a more pronounced gel structure disruption, upon compression, compared to the adsorbed meat proteins which are more sensitive to heat and denature and interact at relatively low temperatures (Feng & Xiong, 2003). Thus, gel structure network formation by SSMP is probably completed at temperatures around 70 °C. According to Feng and Xiong (2003), the presence of soy proteins in meat protein solutions may influence the gel formation process of the latter through possible interactions between the two protein types. Additionally, they suggested that, when soy protein denaturation precedes their incorporation into a gel-forming meat protein solution, the resulting gels exhibit a higher strength. In order to explore this suggestion further, the soy protein solution, constituting the continuous phase of emulsions studied in the present investigation, was heat-treated at 90 °C for 3 min and cooled, to effect protein denaturation. Following emulsification and gel formation the resulting SSMP network strength decreased to a greater extent in the presence of incorporated oil droplets (Fig. 4). This indicates that soy protein denaturation had probably resulted in a decrease of the binding sites on the oil droplet surface for the gel network protein molecules to become attached to. It appears, therefore, that the meat–soy protein interactions may not play a dominant role in determining the strength of these systems.

As Fig. 5 shows, the protein surface load, in emulsions prepared with meat proteins, is significantly lower than that of soy protein emulsions. This may be attributed, by analogy to other proteins, to the fact that part of the soy protein molecules adsorb in the form of aggregates which move to the interface at a higher speed during the homogenization process, resulting in higher protein surface loads compared to more soluble meat protein molecules (Walstra, 1983). The meat proteins, however, possess a higher penetrating power and probably displace a proportion of the adsorbed soy protein aggregates. As appears, from competitive adsorption experiments, that a decrease in the amount of protein load occurs when SPI-stabilized emulsions are equilibrated against a SSMP solution (Fig. 5). Furthermore, as electrophoresis analysis of the adsorbed protein shows, a significant proportion of the protein load is made up of competitively adsorbed meat proteins when an emulsion stabilized with soy protein is allowed to equilibrate against a SSMP solution (Fig. 6). The meat protein constituent appearing to dominate at the interface, following equilibriation of soy protein-stabilized emulsion against a SSMP solution, has a MW corresponding to actin. Other meat protein constituents, on the other hand, are absent, indicating that only actin molecules can penetrate the soy protein-covered oil-water interface and become adsorbed. Since, however,

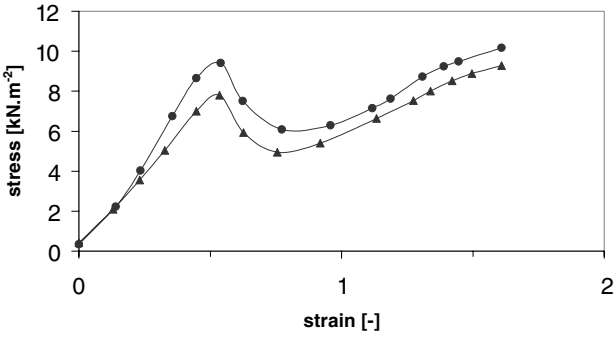

Fig. 4. Stress–strain curves for SSMP gels obtained by heating a 15% SSMP solution at 90 °C for 1 h incorporating 30% emulsified oil with native (●) or denatured (▲) SPI.

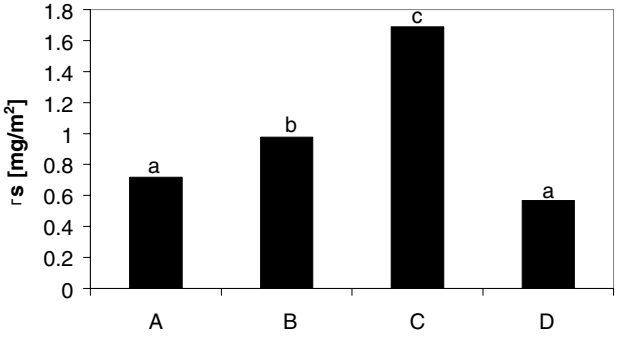

Fig. 5. Protein surface load, Γ_s, of emulsions containing 30% oil and prepared with 1% SSMP (A), with 1% SPI and equilibrated against a 1% SSMP solution (B), with 1% SPI (C), and with 1% mixture of SSMP and SPI (D). [a–c]Different superscripts indicate significant differences at $P < 0.05$.

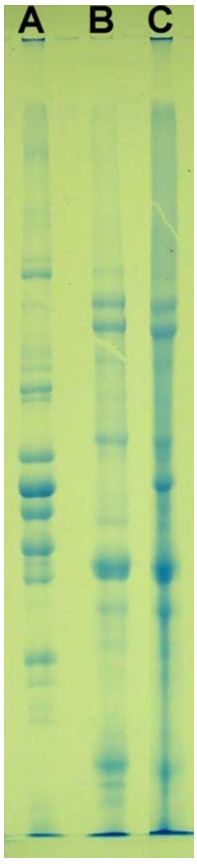

Fig. 6. SDS–PAGE patterns for adsorbed protein of emulsions containing 30% oil and prepared with 1% SSMP (A), with % SPI (B) or with 1% SPI and equilibrated against a 1% SSMP solution (C).

the oil droplet surfaces are covered with a mixture of soy and meat proteins the binding sites available for interaction with the SSMP gel network are probably fewer compared to a system incorporating oil droplets covered with meat proteins only and, therefore, the strength of the gel network is lower.

4. Conclusions

Incorporation of emulsified SSMP or SPI oil droplets into a comminuted meat gel results in network structure disruption, and a lower compressive strength.

The gel structure is envisaged as a composite network where the meat salt-soluble proteins, extracted during the ingredient mixing/cutting process as a result of the presence of NaCl, interact, following heating, with the meat fibres and myofibrils as well as with oil droplet adsorbed-protein bearing surfaces, acting thus as a cementing agent of the system.

Oil droplets covered with SPI interact with the network system through binding sites offered by SSMP molecules, which competitively adsorb at droplet surfaces by displacing loosely adsorbed soy protein molecules.

Acknowledgements

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