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Functional effects of lupin proteins in comminuted meat and emulsion gels

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Abstract

The mechanical and textural properties of gel network resulting by heating at 90 °C of comminuted meat systems, containing lupin seed protein isolate at a relatively low concentration (2%), were studied in an attempt to establish the role of the lupin proteins in the gel structure network development. These results are supported with data from SDS–PAGE analysis of the adsorbed protein at the fat particle surface of the system. The findings are considered in terms of lupin protein involvement in interactions, either at the fat particle surface, or within isolated pockets of high lupin protein content.

Keywords: Lupin protein; Comminuted meat gels; Protein interactions; Meat emulsion; Protein adsorption

1. Introduction

Finely comminuted meat products, such as frankfurters and bologna, mainly depend, for their processing and functional characteristics, on salt soluble myofibrilar proteins which, following extraction during ingredient mixing in the presence of salt, adsorb at the product fat particle surfaces, due to their remarkable emulsifying ability. At the same time, these proteins coagulate during processing and/or cooking, resulting in the formation of a gel-like structure that binds muscle fibrils with the emulsified fat particles, thus preventing liquid losses and contributing to the development of the final product texture (Acton & Dick, 1989; Barbut, 1995).

Proteins of plant origin, especially those of soybean, are often incorporated in meat product batters to improve their processing and/or cooking behaviour and also final product properties, especially their mechanical and textural characteristics (Barbut, 1994; Rhee, 1994). Proteins, extracted from lupin seed in the form of isolate, present an interesting alternative to soybean proteins in meat products, as recent research has demonstrated (Alamanou, Doxastakis, Bloukas, & Paneras, 1996; Mavrakis, Doxastakis, & Kiosseoglou, 2003). Lupin seeds are rich in protein that presents a good balance of essential amino acids. The high protein concentration is sometimes superior to that of soybean and, additionally, lupin protein amino acid pattern is comparable to that of soybean (Cerletti & Duranti, 1979). Lupin proteins possess important emulsifying properties (Pozani, Doxastakis, & Kiosseoglou, 2002) and are expected to contribute to the stabilization of fat particles in a comminuted meat product system. Additionally, their gel-forming ability (Kiosseoglou, Doxastakis, Alevisopoulos, & Kasapis, 1999; Mavrakis et al., 2003) allows them to strengthen the structure of a processed/cooked product.

The way the proteins of plant origin function in a meat product system, however, is far from clear. Although these proteins possess important functional properties and there are a number of research papers reporting that the soy protein constituents may interact with the meat proteins in processed meat products (Feng & Xiong, 2002; Peng & Nielsen, 1986; Xiong, 1997), it appears that the properties

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of the system are dominated by the more functional salt-soluble meat proteins while the plant proteins play a rather secondary role (Mourtzinos & Kiosseoglou, 2005). It remains a fact, however, that the large deformation properties of model comminuted meat product systems are enhanced by the presence of plant proteins, such as lupin seed proteins, indicating that these proteins are involved in the development of the multicomponent comminuted meat gel structure during processing (Mavrakis et al., 2003).

This investigation was undertaken to probe the way the lupin seed proteins contribute to the strengthening of the comminuted meat product structure, considering that LSPI addition at a relatively low level results in an increase of fracture parameter values of meat gels (Mavrakis et al., 2003) while their adsorption at the product fat particle surfaces, in the presence of salt-soluble meat proteins, has not been reported up to now.

2. Materials and methods

2.1. Materials

After grinding through a 4.5 mm plate, comminuted lean pork meat and back fat were obtained from a local meat market and, following packing in moisture-proof bags, the samples were frozen at −20 °C for 1 week before use. Lupin seed protein isolate samples (LSPI-Type E) were provided by the Fraunhofer Institute (Freising, Germany). The isolates differed in the air temperature conditions applied for dehydrating (in the spray-drier) the wet lupin seed protein precipitate, which was recovered by isoelectric precipitation at pH 4.5 from a protein extract obtained by dispersing a defatted lupin seed flour in water and adjusting the pH to 8.0. Two isolates exhibiting the highest and the lowest strength (coded LSPI-H and LSPI-L, respectively) were then selected by applying preliminary large compression tests on lupin gel cylindrical samples, and their functional performance was studied further.

2.2. Protein solubility

The LSPI samples were dispersed in distilled water at a concentration of 1% (w/v) in protein and the pH was adjusted to 9.0 by using 1 N NaOH while agitating for 30 min with the aid of a mechanical stirrer. The pH was then reduced to 6.5. Following centrifugation at 3000*g* for 30 min, a clear protein solution was obtained and its protein content was analyzed by applying the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951).

2.3. Lupin protein and comminuted meat gel preparation

Lupin protein gels were prepared by first dispersing the isolate samples in phosphate buffer (1/15 M Na_2HPO_4, 1/15 M KH_2PO_4) of pH 6.5, by continuously stirring with the aid of a magnetic stirrer, to obtain a dispersion of 20% (w/w) in lupin protein. The protein dispersions were then poured into cylindrical cells of 1 cm diameter, constructed from aluminium foil and reinforced with plastic tape. The ends of the cells were then tightly sealed to prevent leakage and the cells were heated in a water bath at 90 °C for 1 h, followed by storage at room temperature for 24 h. The cells were finally opened and cylindrical samples of 1 cm in height were cut from the gels.

To prepare model comminuted meat gels, 100 g of partially defrosted comminuted lean meat were mixed with 50 g ice and 3.5 g NaCl (CM-A), or with 12 g ice and 0.6 g NaCl (CM-B), using a blender fitted with knives, operating at low speed. Following storage of the resulting meat paste at 4 °C for 24 h, to effect extraction of the salt-soluble meat proteins, and addition of 20% LSPI dispersions in protein, to obtain a comminuted meat paste of 2% in plant protein, cylindrical cells were again filled and gel preparation was performed as described above. Model comminuted meat gel samples containing 25% pork fat were prepared in the same way from a paste made up of 50 g lean meat, 25 g ice, 25 g fat and 1.75 g NaCl.

2.4. Measurement of gel fracture and textural properties

A Stable Micro System TA-XT2i Texture Analyzer, equipped with 2.5 cm diameter compression cylinder, was employed to determine the fracture and textural parameter values of the cylindrical gel samples which were subjected to 80% compression at a constant speed of 0.5 mm/s at room temperature. The apparatus plates were lubricated with vegetable oil to avoid bulging of the middle part of the cylindrical sample and the compression force–time curves were analyzed by using the following equations (Van Vliet, 1999):

$$G(\mathrm{t}) = \frac{F_t}{A_t} \tag{1}$$

and

$$E_{\mathrm{H}} = \ln\frac{L_t}{L_{\mathrm{o}}}, \tag{2}$$

where $G(t)$ and E_{H} are the stress and the Hencky strain, $F(t)$ is the force at time t, $A(t)$ is the surface area of the test sample calculated from:

$$A(\mathrm{t}) = \frac{L_{\mathrm{o}}}{L_t}A_{\mathrm{o}}, \tag{3}$$

where L_{o} and L_t are the original height and the height after deformation time, t, respectively.

To determine the texture parameter values of gels, the cylindrical samples were subjected to double compression (50% compression at a speed of 0.5 mm/s) and the resulting curves were analyzed according to the texture profile analysis method (Bourne, 1978) with the aid of the Stable Micro System Expert software programme.

2.5. Measurement of liquid loss during cooking or upon compression

Following cooking, the gels were cut open and the liquid loss during gel preparation was determined by weighing while the ability of the gel samples to retain liquid under pressure was determined, by applying the modified procedure proposed by Funami, Yada, and Nakao (1998), from the liquid lost from a 2 mm thick cylindrical sample subjected to 10% compression for 30 min between layers of filter paper with the aid of the Textural Analyzer.

2.6. Protein adsorption at fat surfaces

Following preparation of the comminuted meat paste and storage at 4 °C for 24 h, 5 g of the paste were dispersed in 100 ml of 0.6 M NaCl solution and stirred with the aid of a magnetic stirrer for 1 h to liberate the fat which was separated by centrifugation at 5000*g* for 30 min. The fat layer was then recovered from the top of the centrifuged sample and subjected to repeated washing steps with a 0.6 M NaCl solution.

The fat particles were then stained in a 0.1% solution of R250 Coomassie brilliant blue and photographed with the aid of a Kodak camera. The protein adsorbed on fat particles was extracted with the electrophoresis sample buffer (25% (v/v) Tris–SDS pH 6.8; 2% (w/v) SDS; 10% (v/v) glycerol; 5% (v/v) 2-mercaptoethanol; 0.1% (v/v) bromophenol blue) and analyzed by SDS–PAGE, as described by Laemmli (1970) with the help of an Apelex Model ST 1006T vertical electrophoresis apparatus (Scie-Plas, Warwickshire, England) using 3% and 10% acrylamide stacking and resolving gels, respectively.

2.7. Statistical analysis

All experiments were repeated at least three times and the data were analyzed using the one-way ANOVA programme. The level of confidence was 95%. Significant differences between means were identified by the LSD procedure.

3. Results and discussion

As Fig. 1 shows the resistance to compression of gels prepared by heating LSPI dispersions depended on spray drier air temperature with the sample dried at an initial air temperature of 210 °C producing a gel network structure that yielded at a much higher stress value than that dried at 175 °C. Although this difference between the two gel systems is difficult to explain, as both LSPI samples exhibited similar protein solubility values (around 67–69% (w/w)), one may hypothesize that the higher initial air temperature in the drier, that resulted in an increase of the drying rate, could have affected protein-protein interactions in the dehydrated isolate and possibly its gel network structure-forming characteristics.

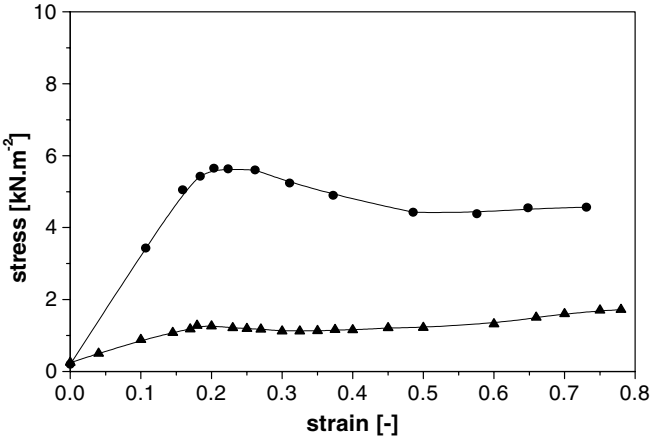

Fig. 1. Stress–strain curves for 20% (w/w) LSPI-H (●) and LSPI-L (▲) gels.

When the lupin protein isolates were incorporated in comminuted meat paste systems the resulting gels, obtained by heating at 90 °C, exhibited higher stress values, the effect of the presence of lupin seed protein depending on parameters such as water, salt or fat content as well as drying air temperature conditions (Fig. 2). It is noteworthy that, when the amount of water and the salt content decreased, the influence of lupin protein on the gel fracture parameter values increased (Fig. 3) while the liquid losses upon compression were less extensive (Table 1). This suggests that the contribution of lupin proteins to the development of the gel network structure became more pronounced.

Lupin isolates tend to form gel networks at a protein concentration above 13%, the critical gel protein content depending on parameters such as the type of isolate, the NaCl concentration and the final heating temperature (Mavrakis et al., 2003). The lupin protein content of the comminuted meat systems studied in the present work was below the critical gel point, if it is assumed that the water content of the lean muscle is about 70% (Herbert, 1985). One may, therefore, hypothesize that a gel network formation by lupin protein molecules should not have taken place in a system dominated by salt-soluble meat proteins which, due to their much lower denaturation temperature compared to lupin proteins (80–90 °C; Mavrakis et al., 2003), tend to become involved in gel network formation while the lupin proteins are excluded from the system in isolated pockets within the gel matrix, where they remain in the dispersed form. This model, however, does not explain the remarkable structure-strengthening effect of lupin proteins in comminuted meat gel systems. According to Mavrakis et al. (2003), this effect should have been the result of the high emulsifying properties of lupin proteins which, following adsorption at the lipid particle surfaces, may contribute to the formation and the strengthening of the meat gel structure. To test this hypothesis, the protein adsorbed on the surfaces of fat particles of comminuted meat emulsions

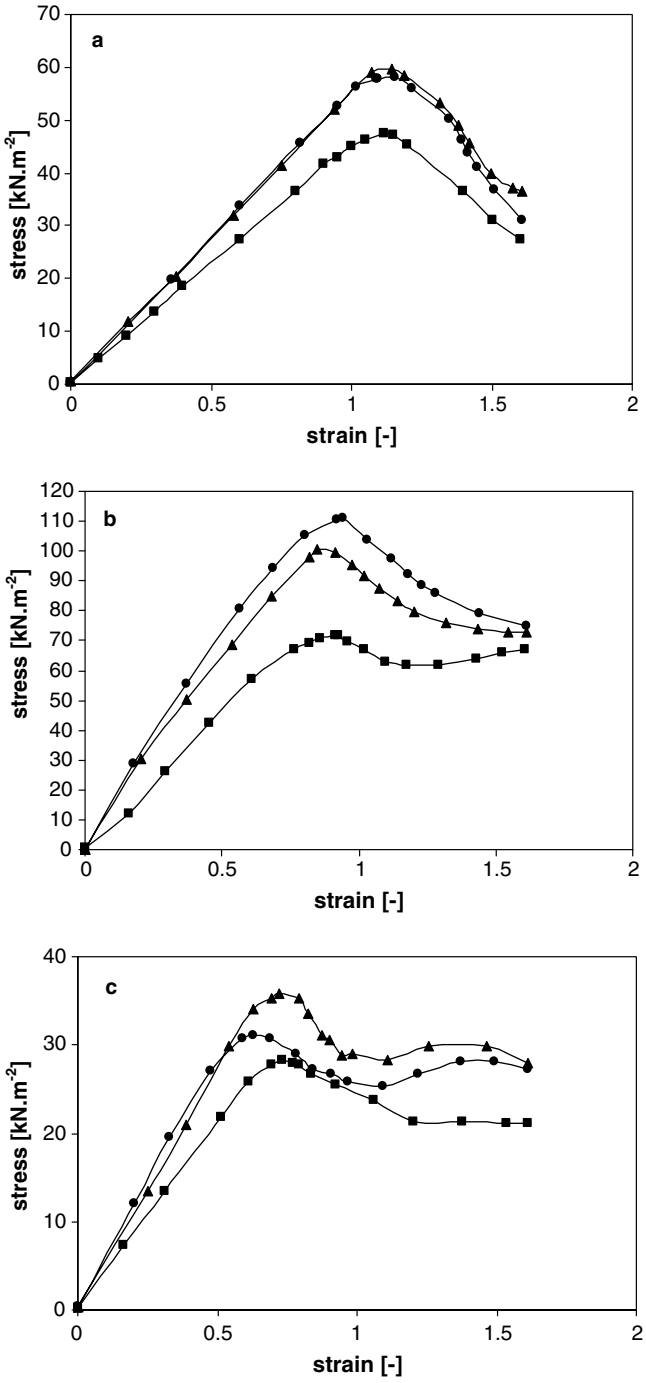

Fig. 2. Stress–strain curves for gels prepared by heating comminuted meat paste CM-A (a), CM-B (b), or CM-A containing 25% fat (c). Key: (■) control; (●) LSPI-H; (▲) LSPI-L.

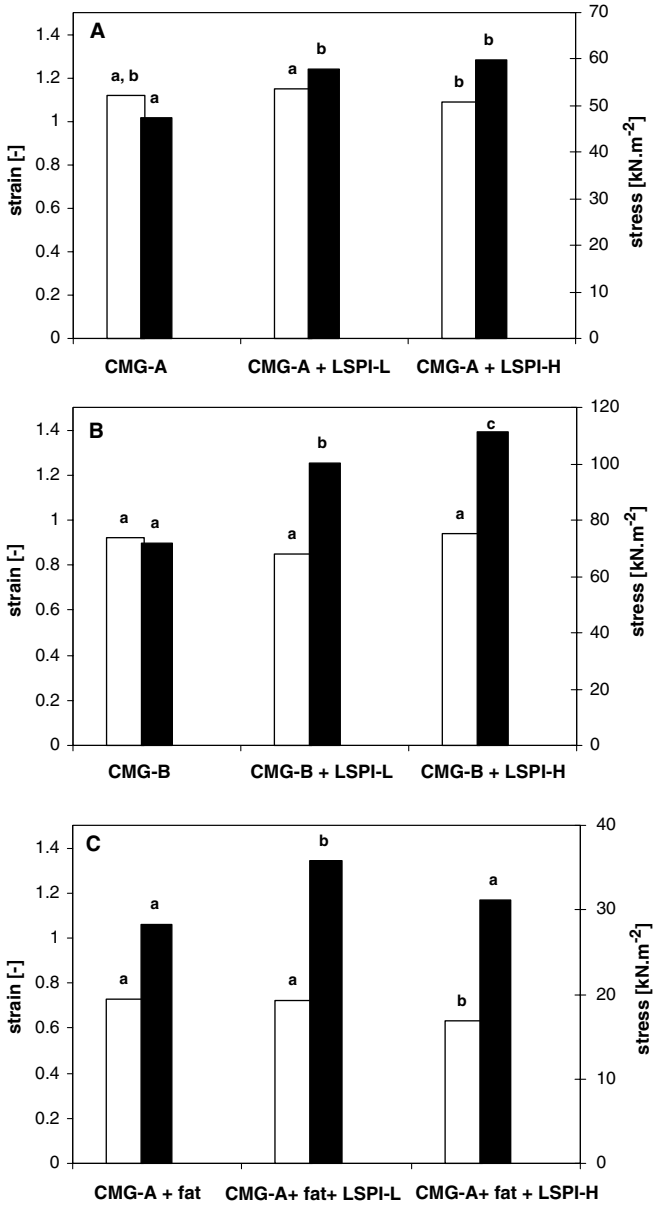

Fig. 3. Fracture stress and strain parameter values for gels prepared by heating comminuted meat paste CM-A (A), CM-B (B), or CM-A containing 25% fat (C). Key: (■) stress; (□) strain. [a–c]Different superscripts indicate significant differences at $P < 0.05$.

was recovered and analyzed by SDS–PAGE. As shown in Fig. 4, the surface of fat is uniformly covered with protein, although patches of higher colour density are also spotted, suggesting that some protein aggregates may also have become adsorbed along with isolated protein molecules. According to Fig. 5, some lupin protein constituents may have become involved in the formation of the surface membrane of the fat particles, although it appears that this surface was dominated by the muscle proteins. It is more plausible then to assume that the comminuted meat gel strengthening effect of lupin proteins could have been rather connected with their involvement in gel structure formation.

Following the salt-soluble gel network formation during heating, the lupin proteins are expected to remain unreacted and concentrate within isolated pockets of the gel system. As the temperature is further increased, denaturation and lupin protein interaction may take place within these isolated pocket areas. Such interaction may also take place at the surfaces, where the lupin protein-rich phase

Table 1
Effect of 2% (w/w) LSPI-H or LSPI-L incorporated in comminuted meat gels in the absence or presence of 25% fat, on liquid loss (U) during cooking or upon compression

Sample	Heating	Compression
CMG-A[A]	3.27[a]	18.75[a,b]
CMG-A containing 2% (w/w) LSPI-L	3.35[a]	19.07[a]
CMG-A containing 2% (w/w) LSPI-H	4.24[b]	17.73[b]
CMG-A (25% fat)	8.71[a]	16.05[a]
CMG-A (25% fat) containing 2% (w/w) LSPI-L	7.10[b]	14.74[b]
CMG-A (25% fat) containing 2% (w/w) LSPI-H	7.17[b]	15.46[a]
CMG-B[B]	5.29[a]	16.52[a]
CMG-B containing 2% (w/w) LSPI-L	4.07[b]	12.81[b]
CMG-B containing 2% (w/w) LSPI-H	4.47[c]	13.56[b]

[a–c] Different superscripts within each group of samples indicate significant differences at $P < 0.05$.
[A] CMG-A: Comminuted meat gel with 50 g ice and 3.5 g NaCl.
[B] CMG-B: Comminuted meat gel with 12 g ice and 0.6 g NaCl.

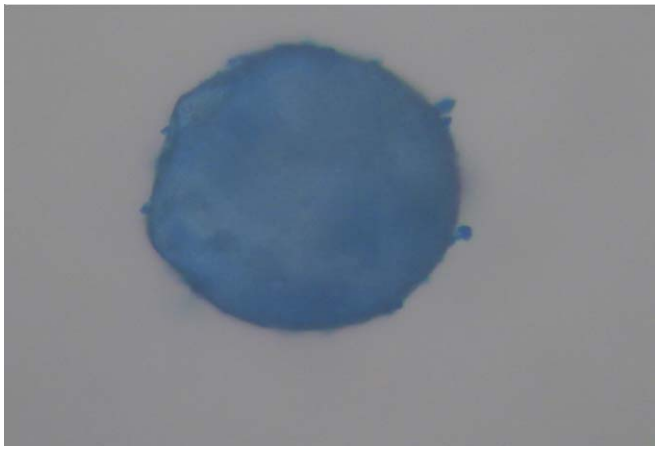

Fig. 4. Fat particle isolated from comminuted meat paste containing 25% fat and 2% (w/w) LSPI-H and stained with Coomassie brilliant blue R250.

meets the salt-soluble meat proteins. Interactions of meat proteins with proteins of plant origin, such as soy proteins, have been reported by Peng and Nielsen (1986) and also by Feng and Xiong (2002), when mixtures of the two proteins are heated at temperatures exceeding the denaturation points of the proteins of the mixture. Furthermore, if we assume that a large part of the water of the meat is entrapped within the myofibrils as well as in the gel network, it is highly probable that the water content within the lupin protein-rich areas could have been so low as to bring about an increase in the effective lupin protein content in these areas, allowing the proteins to interact and produce a second gel structure that acts as a filler for the primary meat protein gel network. Thus, the isolated lupin gel areas may lead to the strengthening of the structure, irrespective of the presence or absence of fat particles, although the involvement of adsorbed lupin protein molecules at the particle surfaces in structure development cannot be excluded.

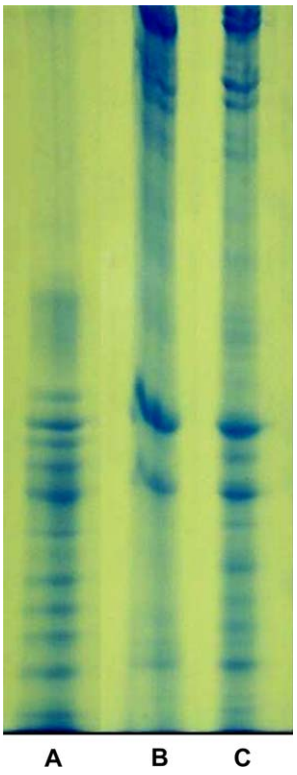

Fig. 5. SDS–PAGE patterns for adsorbed protein on fat particles of comminuted meat paste in the absence (B) or presence of 2% (w/w) LSPI-H (C). (A) corresponds to LSPI-H.

4. Conclusions

Incorporation of LSPI in comminuted meat paste and heating at 90 °C results in an increase of the gel network resistance to compression, the result depending on composition with respect to fat, water and salt. Although the lupin proteins tend to adsorb at the fat particle surfaces of the comminuted meat system, these surfaces are dominated by the salt-soluble meat proteins of meat. The strengthening effect of lupin proteins is attributed to their increasing effective content, resulting from a gel structure formation by the meat myofibrils and the extracted salt-soluble meat proteins, that enhances protein interactions between the lupin proteins.

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