ELSEVIER

Food Chemistry 85 (2004) 423–429

Food Chemistry

www.elsevier.com/locate/foodchem

Transglutaminase as binding agent in fresh restructured beef steak with added walnuts

A. Serrano, S. Cofrades, F. Jiménez Colmenero*

Instituto del Frío (CSIC). C/José Antonio Novais, 10. Ciudad Universitaria, 28040, Madrid, Spain

Received 25 April 2003; received in revised form 21 July 2003; accepted 21 July 2003

Abstract

Restructured beef steaks with added walnuts (0, 10 and 20%) and salts (2% NaCl and 0.3% sodium tripolyphosphate), were prepared, using microbial transglutaminase (MTG) (0.7%)/sodium caseinate (3%) as cold-set binder, and stored in chilling conditions (6 days at 3 °C). Restructured beef steak with walnuts presented acceptable sensory characteristics. Addition of both walnuts and salts reduced the total loss (sum of purge and cooking losses), which increased ($P<0.05$) during storage. Added walnut reduced ($P<0.05$) binding strength of uncooked and cooked products. With MTG, the restructured beef steaks presented suitable mechanical characteristics (meat particle binding) for handling in the raw state. However, other means need to be used, along with MTG, to induce the protein–water interactions required for suitable water and binding properties in fresh and cooked products.

Keywords: Restructured; Beef steak; Transglutaminase; Walnut; Chilling storage

1. Introduction

Cardiovascular diseases are the principal cause of death in developed countries, and diet is one of the major factors in their incidence. Epidemiological studies show that frequent consumption of nuts in general, and walnuts in particular, correlates inversely with myocardial infarction or death by vascular ischaemic disease, regardless of other risk factors, such as age, sex, smoking, hypertension, weight and exercise (Fraser, Sabaté, Beeson, & Strahan, 1992; Iwamoto et al., 2000; Sabaté, 1993). This effect has been associated with the peculiar blend of nutrients and phytochemical compounds found in walnuts: high-biological-value proteins, vegetable fibre, polyunsaturated (linoleic and linolenic) fatty acids and micronutrients, such as folic acid, magnesium, liposoluble vitamins (especially γ-tocopherol), and other antioxidants (phytosterols and polyphenols). All these combine in complex ways to exert beneficial effects on serum lipid profiles and other risk factors that can cause or exacerbate cardiovascular diseases.

Restructured meats offer many major advantages for consumers and for the meat industry. Experiments using conventional meat restructuring systems (including NaCl and phosphate) with added walnuts to make restructured beef steak (precooked and frozen) have resulted in products with acceptable physicochemical and sensory properties (Cofrades, Serrano, Ayo, Solas, Carballo, & Jiménez Colmenero, in press; Jiménez Colmenero, Serrano, Ayo, Solas, Cofrades, & Carballo, in press). Such kinds of meat products can only be marketed either precooked or frozen because the product bind is not very strong in the raw state. However, consumers tend to appreciate these less than fresh meat products, and they therefore need to be suitable for raw handling in the chilled state (Kuraishi, Sakamoto, Yamazani, Susa, Kuhara, & Soeda, 1997; Wijngaards & Paardekooper, 1988).

The use of transglutaminase has been described as a procedure for cold gelification of muscle protein which can reduce or eliminate the need to add NaCl and phosphate (Kuraishi et al., 1997; Nielsen, Petersen, & Moller, 1995; Wijngaards & Paardekooper, 1988). Because of the potential health benefits, there is growing interest among consumers and processors in reducing the use of NaCl in meat processing. Kuraishi et al.

* Corresponding author. Fax: +34-91-549-36-27.
E-mail address: fjimenez@if.csic.es (F. Jiménez Colmenero).

0308-8146/$ - see front matter
doi:10.1016/j.foodchem.2003.07.021

(1997) reported that a microbial transglutaminase (MTG)/sodium caseinate (C) system (0.05–0.1% MTG/ 0.5–1% C) could be usefully employed as a meat binder at low temperature (5 °C). With this system, transglutaminase could serve as a real cold-set binder to produce restructured meat in the raw, refrigerated state without addition of NaCl. However, this study reported no data on how this treatment might affect important product characteristics, such as water-binding properties in the raw state and water-binding and textural properties of cooked muscle food.

For practical application, the effect of a MTG/C system on restructured meat prepared for distribution as a chilled product has to be assessed over several days to reflect real commercial conditions. However, there are hardly any studies on how the time in chilled storage can affect the characteristics of raw and cooked meat systems prepared with MTG as a cold-set binder (Carballo, Ayo, & Jiménez Colmenero, submitted for publication). Carballo et al. reported changes in characteristics during chilling storage of finely comminuted meat batter (from different meat species) prepared with MTG. However, no information is available on other studies that provide data on the behaviour of fresh restructured steak treated with MTG/G (and processed at less than 10 °C) after several days in chilled storage.

One purpose of this experiment was to ascertain how use of a TGM/C system as a cold-set binder affected the characteristics of restructured beef steak with different concentrations of walnut (0, 10 and 20%). Another was to determine the influence of chilling storage (6 days at 3 °C) on the properties of these meat products. The parameters measured to determine the effect of the treatment were the sensory, colour and water/fat and meat particle binding properties of raw and cooked products.

2. Materials and methods

2.1. Meat preparation and additives

Select beef top rounds (15 kg) were trimmed of fat and connective tissue and cut into strips (approx. 5×4×20 cm). Lots of approx. 1.2 kg, were vacuum-packed, frozen to −20 °C and stored at that temperature until used.

The following additives were used for preparation of restructured beef: sodium caseinate (Anvisa, Arganda del Rey, Madrid, Spain), microbial transglutaminase (Activa WM, Ajinomoto Co, Inc, Kawasaki, Japan), sodium chloride (Panreac Quimica, S.A. Barcelona, Spain) and sodium tripolyphosphate (STP) (Panreac Quimica, S.A. Barcelona, Spain). Walnut, ground down to a particle size of <0.8 mm, was supplied by Bernardo Josa Quilez, (Valencia, Spain).

2.2. Products preparation

For the preparation of restructured beef steak, meat packages were thawed (approx. 18 h 3±2 °C, reaching between −3 and −5 °C) and passed once through a grinder (Mainca, Granollers, Spain) with a 2-cm plate. Four different products were formulated (Table 1). The procedure was as follows: meat was mixed for 1 min in a mixer (Mainca, Granollers, Spain); half of the water (with salt and STP as appropiate) was added and the whole mixed again for 1 min; sodium caseinate was sprinkled on and the whole mixed again for 2 min. Transglutaminase was dissolved in the other half of the water with an Omnimixer (Omni International, Waterbury, CT USA); this solution was added to the first and the whole mixed again for 2 min. Finally, walnut was added and the whole mixed again for 2 min. Mixing time was standardized at 8 min.

Each batch was placed in metal moulds (1.25 kg) and stored at 3 °C overnight, to allow MTG action. Metal moulds were kept at −18 °C until the samples were solid enough to be sliced (Mainca, Granollers, Spain) into steaks (140 g±3; 1.0 cm±0.05 thick). Each steak was weighed, vacuum packed and stored at 3 °C. Evaluations were performed initially (16–18 h after packaging), and at 2 and 6 days.

2.3. Proximate analysis and pH

Moisture and ash contents of the raw samples were determined (AOAC, 1984) in quadruplicate. Fat content was evaluated (in duplicate) according to Bligh and Dyer (1959). Protein content was measured in quadruplicate by a Nitrogen Determinator LECO FP-2000 (Leco Corporation, St. Joseph, MI). The pH of the raw products was determined in duplicate using a pH meter (Radiometer PHM 93, Copenhagen, Denmark) on a homogenate of a 10 g sample in 100 ml distilled water.

2.4. Sensory evaluation

Samples from each formulation were randomly assigned for sensory evaluation. Steaks were cooked in a forced air oven (Rational CM6, Großküchentechnik GmbH, Landsberg a. Lech) (at 170 °C during 10 min) to a core 70 °C determined beforehand by inserting thermocouples, which were connected to a temperature recorder (Yokogawa Hokushin Electric YEW, model 3087, Tokyo, Japan). Slices were cut into uniform sized pieces and served warm (~45 °C) to a 10 member panel. Panellists were selected from among staff in a preliminary session, then trained with the products and terminology. A description of attributes was discussed with the panel members. Panellists were asked to evaluate the samples on a non-structured scale (of 10 cm) without fixed extremes, with reference to the following

Table 1
Formulation of different restructured beef steaks[a]

Samples	Beef (g)	NaCl (g) + STP (g)	Walnut (g)	Water (g)	MTG (g)	Caseinate (g)	Total (g)
NS/0	3452	0	0	400	28	120	4000
NS/10	3052	0	400	400	28	120	4000
NS/20	2652	0	800	400	28	120	4000
S/10	2960	80 + 12	400	400	28	120	4000

[a] STP, sodium tripolyphosphate; MTG, microbial transglutaminase. The first term in each sample denomination indicates: NS, salt-free products; S, product prepared with added salts (NaCl and STP). The second indicates added walnuts (%).

parameters: flavour, texture, and overall acceptability. Each point marked was converted to a numerical value from 0 (dislike extremely) to 10 (like extremely) according to location. At the same time panellists were asked to evaluate the off-flavour according to the following scale: 1 (none) and 5 (intense). Sensory evaluation was conducted under red light to minimize perceptions of colour caused by the different proportions of walnut. Each panellist tasted three samples per session.

2.5. Surface colour

Surface colour (lightness, L; redness, a and yellowness, b) of raw restructured beef steaks was evaluated on a HunterLab model D25-9 (D45/2°) (Hunter Associates Laboratory Inc., Reston, VA). Colour determinations were performed immediately before bind strength analysis on the beef steaks, whole and 20 min after the packages were opened. Determinations were performed on three steaks per formulation; there were six determinations per steak (three on either surface of the steak).

2.6. Purge loss, cooking loss and total loss

Three restructured beef steaks from each formulation were removed each time (initial, 2 and 6 days) from their vacuum packages and after 15 min were manually wiped with a paper towel to remove visible exudate. Purge loss (PL) was calculated as weight loss divided by original weight (before packaging), expressed as a percentage.

These three restructured beef steaks were cooked in a forced air oven (Rational CM6, Großküchentechnik GmbH, Landsberg a. Lech) (at 170 °C during 10 min) to a core 70 °C determined beforehand by inserting thermocouples, which were connected to a temperature recorder (Yokogawa Hokushin Electric YEW, model 3087, Tokyo, Japan). After 30 min at room temperature (20–22 °C), steaks were manually wiped with a paper towel to remove visible exudate. Cooking loss (CL) was calculated as weight loss divided by original weight (before packaging), expressed as a percentage. Total loss (TL) was calculated as the sum of purge and cooking loss. These parameters reflect water- and fat-binding properties of the samples.

2.7. Binding strength

Portions were prepared from three raw restructured beef steaks per formulation. Six portions (5×5×1 cm) were obtained per formulation to assess the ability of the meat pieces to adhere to one another (bind strength), in a procedure similar to that of Field, Williams, Prasad, Cross, Secrit, and Brewer (1984). The bind strength (BS) was measured as the peak force (N) required for a 1.9 cm ball, at a cross head speed of 100 mm/min (in a 500 N load cell), to break through a meat slice mounted on a ring of 3.2 cm inner diameter.

Similar instrumental texture analyses were carried out on identical cooked portions cut from restructured steaks used to determine cooking loss. Instrumental texture analysis was conducted using a TA-XT2 Texture Analyser (Texture Technologies Corp., Scarsdale, NY). Raw samples and cooked samples were measured at room temperature.

2.8. Statistical analysis

Data were analysed using Statgraphics 2.1 (STSC Inc., Rockville, MD) for one- and two-way ANOVA. The least squares difference method was used to compare mean values among treatments and the Tukey HSD test was used to identify significant differences ($P<0.05$) among main effects (walnut proportion and time of storage).

3. Results and discussion

3.1. Proximate analysis and pH

The proportion of added walnut significantly affected the proximate analysis of restructured beef steak (Table 2). Addition of walnut gradually increased ($P<0.05$) fat values and reduced ($P<0.05$) moisture and protein values (Table 2). The proportion of ash in

S/10 samples was greater, due to the addition of salts. These results are consistent with meat product formulations (Table 1) where meat was replaced by walnut. Both non-meat ingredients (walnut and salts) increased ($P<0.05$) pH of meat products (Table 2). Jiménez Colmenero et al. (2003) reported no effect of walnut on pH.

3.2. Sensory analysis

Sensory evaluation indicated that walnut significantly affected the sensory quality of restructured steak (Table 3). The panel were able to detect the addition of walnut as a slight off-flavour (walnut-like) which was more noticeable ($P<0.05$) the higher the percentage of added walnut. The panel viewed this positively since the flavour, texture and overall acceptability scores were higher than the control. (Table 3). Similar results have been reported by Jiménez Colmenero et al. (2003). The presence of salts (NS/10 versus S/10) produced no appreciable change in the sensory evaluation. It was concluded that restructuring with walnuts produced acceptable sensory characteristics.

Table 2
Proximate analysis (%) and pH of raw restructured beef steaks[a]

Samples	Moisture	Protein	Fat	Ash	pH
NS/0	74.0a	22.2a	2.2a	1.0a	5.7a
NS/10	67.7b	20.8b	8.3b	1.2b	5.9b
NS/20	60.8c	19.2c	13.6c	1.1ab	6.0c
S/10	66.6d	20.7b	8.3b	3.5c	6.1d
SEM	0.17	0.13	0.28	0.03	0.01

[a] For sample denomination see Table 1. Different letters in the same column indicate significant differences ($P<0.05$). SEM = Standard error of the mean.

Table 3
Sensorial analysis of restructured beef steaks[a]

Samples	Flavour	Off-flavour	Texture	Overall acceptability
NS/0	2.4a	1.1a	2.3a	1.9a
NS/10	6.2b	2.3b	6.5b	6.2b
NS/20	5.4b	3.8c	5.7b	5.7b
S/10	5.5b	2.7b	4.2b	5.3b
SEM	0.56	0.21	0.58	0.61

[a] For sample denomination see Table 1. Different numbers in the same column indicate significant differences ($P<0.05$). SEM = Standard error of the mean. Flavour, texture and overall acceptability scale (0: dislike extremely and 10: like extremely). Off-flavour scale (1: none and 5: intense).

3.3. Surface colour

Addition of walnut produced an increase ($P<0.05$) of L and b and a decrease ($P<0.05$) of redness (Table 4). Similar results have been reported by Jiménez Colmenero et al. (2003). In general, salts decreased lightness and increased redness (Table 4). Trout, Chen, and Dale (1990) reported that the presence of NaCl and STP did not affect L, a and b values of restructured pork chops.

Storage time decreased lightness and increased redness, except in the case of the S/10 sample (Table 4). While yellowness of NS/0 and NS/10 samples had increased ($P<0.05$) by day 6 of storage, NS/20 and S/10 were unaffected ($P>0.05$) by refrigerated storage. Other authors (Boles & Shand, 1999; Bradford, Huffman, Egbert, & Jones, 1993) have reported that refrigerated storage of fresh pork sausage patties or restructured beef resulted in decreased lightness, redness and yellowness, although this effect was attributed to extended exposure of products to retail lighting conditions.

3.4. Purge loss, cooking loss and total loss

The presence of walnut reduced ($P<0.05$) purge loss in restructured products over the entire storage period (Table 5). At the outset of storage, purge loss was five times greater in samples without walnut (NS/0) than in samples with 20% walnut (NS/20). Purge loss increased ($P<0.05$) during chilling storage, and after 6 days the difference attributable to walnut was smaller (Table 5). Purge loss (<1%) was smallest ($P<0.05$) when salts

Table 4
Colour parameters of raw restructured beef steaks[a]

	Lightness (L)			Redness (a)			Yellowness (b)		
	Days in storage (3 °C)			Days in storage (3 °C)			Days in storage (3 °C)		
	Initial	2	6	Initial	2	6	Initial	2	6
NS/0	40.65a14	40.13a1	38.71b12	8.92a1	11.62b1	17.46c1	11.73a1	11.42a1	14.66b1
NS/10	44.10a2	43.19a2	39.44b1	8.38a12	12.68b2	11.81b2	13.32a2	13.54a2	14.25b1
NS/20	45.20a3	45.33a3	39.42b1	7.78a2	10.52b3	11.47c2	14.39a3	14.47a3	14.34a1
S/10	41.10a4	41.69a4	38.23b2	12.94a3	7.07b4	11.25c2	13.49a2	12.84a4	12.85a2
SEM		0.26			0.23			0.17	

[a] For sample denomination see Table 1. Different letters in the same row and different number in the same column indicate significant differences ($P<0.05$). SEM = Standard error of the mean.

Table 5
Water- and fat-binding properties of restructured beef steaks[a]

	Purge loss (%)			Cooking loss (%)			Total loss (%)		
	Days of storage (3 °C)			Days of storage (3 °C)			Days of storage (3 °C)		
	Initial	2	6	Initial	2	6	Initial	2	6
NS/0	11.98a1	13.22a1	15.72b1	23.94a1	25.53a1	25.06a1	35.93a1	38.76ªb1	40.78b1
NS/10	5.96a2	6.04a2	10.39b2	21.42a1	21.11a2	20.95a2	27.39a2	27.16a2	31.35b2
NS/20	2.43a3	2.29a3	7.48b3	16.63a2	17.02a3	16.79a3	19.06a3	19.33a3	23.27b3
S/10	0.52a4	0.51a4	0.70a4	8.27a3	10.13a4	9.98a4	8.79a4	10.64a4	10.68a4
SEM		0.41			0.76			0.81	

[a] For sample denomination see Table 1. Different letters in the same row and different numbers in the same column indicate significant differences ($P<0.05$). SEM = Standard error of the mean.

were added (NS/10 versus S/10), and purge loss values remained stable ($P>0.05$) throughout storage (Table 5). Devatkal and Mendiratta (2001) reported up to 10% moisture loss in refrigerated restructured pork rolls (15 days at 4 °C). Fluid accumulation during chilled retail storage is one of the main problems with fresh marketed products as it not only makes the product look unpleasant to the consumer but it can also favour growth of microorganisms. There is little information available on restructured beef steak, but levels around 3.5–4.5%, which have been found in other commercially available meat products, tend to spoil their appearance (López-Caballero, Carballo, & Jiménez Colmenero, 1999).

Addition of walnut generally reduced ($P<0.05$) cooking loss (Table 5), which was unaffected ($P>0.05$) by chilling storage, irrespective of the treatment. As in the case of purge loss, cooking loss was lower in products containing salts at all stages of the experiment (Table 5). The total loss (PL + CL), directly related to PL, decreased ($P<0.05$) in the presence of walnut and increased ($P<0.05$) during storage. Water- and fat-binding properties observed in this experiment (8–40%) are comparable to those reported in similar products (Boles & Shand, 1999; Chen & Trout, 1991; Sheard, Nute, & Chappel, 1998) with reference to a number of different variables (e.g. composition, additives, cooking methods, oven temperature and sample dimensions).

The effect of walnut on these properties may be related to the relative moisture in the samples (Table 2). Our results show that when MTG/C (no salts) was used, the meat products exhibited less water- and fat-binding ability (total loss 35–40%) (Table 5). Various studies have shown that the use of MTG (without salts) can result in meat products with poor water-binding properties. O'Kennedy (2000) reported that the inclusion of MTG alone in a pork meat dispersion had no effect on cooking loss, but when added with sodium caseinate it led to a decrease in cooking loss (40 to 27%). Kerry, O'Donnell, Brown, Kerry, and Buckley (1999) found that the presence of MTG had no influence on high cooking loss (40%) in comminuted poultry products. Meat batters (pork and beef) with MTG/C presented higher cooking loss than meat batters containing salts; cooking loss increased in samples with MTG during chilling storage (Carballo et al., submitted for publication).

The good water-binding properties of the S/10 sample (Table 5) were to be expected since NaCl and STP solubilize meat proteins, which subsequently gel on heating and immobilize water. However, there is some disagreement in the literature as to how the addition of MTG influences the effect of the salts on water-binding (Carballo et al., submitted for publication). The presence of MTG has been reported to have no influence (Kilic, 2003; Pietrasik & Jarmoluk, 2003), to enhance (Kuraishi, Sakamoto, & Soeda, 1996; Pietrasik & Li-Chan, 2002; Tseng, Liu, & Chen, 2000) or to decrease (Carballo et al., submitted for publication; Kuraishi et al., 1996; O'Kennedy, 2000) the effect of salt on water-binding properties of muscle-based products. The effect of MTG on water-binding properties depends on the level and type of MTG used and the conditions in which it is used (e.g. reaction temperature and time, meat particle size and disruption methods, presence of other ingredients and meat source).

Our results suggest that other means need to be used, along with MTG, to induce the protein–water interactions required for suitable water and binding properties in fresh and cooked products. In the present experiment this was achieved by the addition of large amounts of walnut, but most importantly by the addition of salts.

3.5. Binding strength

In salt-free samples, added walnut reduced ($P<0.05$) the binding strength of raw restructured steak (Table 6). Jiménez Colmenero et al. (2003) earlier reported that walnut had no effect on uncooked restructured meat; however, this apparent discrepancy may have been due to the fact that neither the walnut used, nor the gelation process followed to manufacture the products, were the same in this experiment. A comparison of the samples with 10% walnut (NS/10 and S/10) shows that meat particle binding increased ($P<0.05$) in the presence of salts. Chilling storage favoured ($P<0.05$) higher BS in all cases. Increases in penetration force during chilling storage of fresh meat batters (from different species) prepared with MTG as cold-binder agent have likewise been reported (Carballo et al., submitted for publication). Irrespective of treatment, the formulated fresh meat products presented structures suitable for handling in the raw state. Cooking increased the bind strength of restructured meats. The effects of walnut, salts and chilling storage on BS were similar to those recorded for fresh products (Table 6). A decrease in BS with the addition of walnut to cooked restructured steak has been reported elsewhere (Jiménez Colmenero et al., 2003).

The effect of adding walnut on meat particle binding has been associated with a number of factors (Jiménez Colmenero et al., 2003). Increasing fat content (in proportion to walnut concentration) produces softer textures in restructured beef steak (Penfield, Costello, McNeil, & Riemann, 1988). Addition of ingredients also reduces the proportion of water available to form a gel matrix between meat pieces, which could again limit the binding process (Farouk, Hall, & Swan, 2000). Various authors have reported that the addition of some ingredients to meat products produced structures that were less rigid and more easily broken. This behaviour was attributed primarily to the dilution effect of non-meat ingredients in meat protein systems (Rocha-Garza & Zayas, 1996; Tsai, Unklesbay, Unklesbay, & Clarke, 1998) or to their ability to reduce friction and/or binding among meat particles (Saleh & Ahmed, 1998); or again, in relation to cooking yield, it has been suggested that less cooking loss (total loss in this case) makes for products that are less rigid and more easily broken apart during binding valuations (Shao, Avens, Schmidt, & Maga, 1999).

Salt-induced protein solubilization may influence the BS of restructured meats in two ways: directly through the thermal gelation process and indirectly through the action of MTG. Salt has been reported to have a positive effect on the cold-set binding capacity of MTG (S/10 versus NS/10) (Table 6) in uncooked and cooked restructured meats (Carballo et al., submitted for publication; Kuraishi et al., 1997; Tseng et al., 2000). This behaviour has been attributed to the fact that NaCl and STP promote the extraction of myofibrillar proteins, which in turn act as a binding agent and make a good substrate for crosslinking reactions by MTG (Kuraishi et al., 1997) leading to better meat particle binding (Table 6).

There are several factors that can help to explain increasing BS during storage (Table 6). Total weight loss (purge and cooking loss) of restructured meats increased in the course of storage (Table 5), and in such cases the products are generally more difficult to break apart during binding evaluation (Shao et al., 1999). However, this does not seem sufficient to account for the changes in texture of sample S/10. The persistence of residual MTG activity after 24 h would presumably generate additional crosslinking reactions and hence greater binding of meat particles (Carballo et al., submitted for publication). In most available studies, the MTG was allowed to act (reaction times vary from a few hours to around 24 h) and the product was cooked before analysis (Chen, Chou, & Liu, 1998; Kilic, 2003; Pietrasik, 2003; Pietrasik & Li-Chan, 2002; Ruiz-Carrascal & Regenstein, 2002). We therefore have no information on other studies that provide data on the behaviour of fresh meat products treated with MTG (and processed at less than 10 °C) after several days in chilled storage.

Incorporation of nuts in meat products can be used to confer potential heart-healty benefits (Spanish Patent Application 200300367). Restructured beef steak with walnut presented acceptable sensory characteristics. When MTG was used as a cold-binder agent in restructured beef steaks, the products were mechanically suitable (meat particle binding) for handling in the raw state but, when used in fresh and cooked products, water and binding properties were inadequate. Other means are therefore required to improve these properties, including the addition of non-meat ingredients such as walnuts, and more importantly salts.

Table 6
Binding strength (N) of raw and cooked restructured beef steaks[a]

Samples	Raw			Cooked		
	Days in storage (3 °C)			Days in storage (3 °C)		
	Initial	2	6	Initial	2	6
NS/0	4.70a1	6.34b1	8.41c1	20.61ab1	17.53a1	24.06b1
NS/10	3.26a2	4.08ab2	4.50b2	13.21a2	15.93a1	19.87b2
NS/20	2.39a2	2.89ab3	3.49b2	8.71a3	10.42ªb2	13.65b3
S/10	4.70a1	8.60b4	7.58b1	23.87a1	26.52a3	31.37b4
SEM		0.26			0.91	

[a] For sample denomination see Table 1. Different letters in the same row and different numbers in the same column indicate significant differences ($P<0.05$). SEM = Standard error of the mean.

Acknowledgements

This research was supported under project AGL2001-2398-C03-01, Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I + D + I), Ministerio de Ciencia y Tecnología. Thanks are due to Bernardo Josa Quilez for supplying the walnuts.

References

AOAC. (1984). *Official methods of analysis* (14th ed). Washington, DC, USA: Association of Official Analytical Chemists.

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extractions and purification. *Canadian Journal of Biochemistry and Physiology*, *37*, 911–917.

Boles, J. A., & Shand, P. J. (1999). Effects of raw binder system, meat cut and prior freezing on restructured beef. *Meat Science*, *53*(4), 233–239.

Bradford, D. D., Huffman, D. L., Egbert, W. R., & Jones, W. R. (1993). Low-fat fresh pork sausage patty stability in refrigerated storage with potassium lactate. *Journal of Food Science*, *58*(3), 488–491.

Carballo, J., Ayo, J., & Jiménez Colmenero, F. Microbial transglutaminase/caseinate systems as a cold set binders in different meat species. *Meat Science* (submitted for publication).

Chen, C. M., & Trout, G. R. (1991). Sensory, instrumental texture profile, and cooking properties of restructured beef steaks made with various binders. *Journal of Food Science*, *56*(6), 1457–1460.

Chen, M. T., Chou, S. J., & Liu, D. C. (1998). Effect of transglutaminase of chicken liver from different steps of purification on the physical properties of gel from chicken surimi. In *Proceedings 44th International Congress of Meat Science and Technology* (pp. 528–529), 30 August–4 September, Barcelona, Spain.

Cofrades, S., Serrano, A., Ayo, J., Solas, M. T., Carballo, J., & Jiménez Colmenero, F. Restructured beef with different proportions of walnut as affected by meat particle size. *European Food Research and Technology* in press.

Devatkal, S., & Mendiratta, S. K. (2001). Use of calcium lactate with salt-phosphate and alginate-calcium gels in restructured pork rolls. *Meat Science*, *58*(4), 371–379.

Farouk, M. M., Hall, W. K., & Swan, J. E. (2000). Attributes of beef sausages, batters, patties and restructured roasts from two boning systems. *Journal of Muscle Foods*, *11*(3), 197–212.

Fraser, G. E., Sabaté, J., Beeson, W. L., & Strahan, T. M. (1992). A possible protective effect of nut consumption on risk of coronary heart disease. *Archives of Internal Medicine*, *152*(7), 1416–1424.

Field, R. A., Williams, J. C., Prasad, V. S., Cross, H. R., Secrit, J. L., & Brewer, M. S. (1984). An objective measurement for evaluation of bind in restructured lamb roasts. *Journal of Texture Studies*, *15*(2), 173–178.

Iwamoto, M., Sato, M., Kono, M., Hirooka, Y., Sakai, K., Takeshita, A., & Imaiuzumi, K. (2000). Walnuts lower serum cholesterol in Japanese men and women. *Journal of Nutrition*, *130*(2), 171–176.

Jiménez Colmenero, F., Serrano, A., Ayo, J., Solas, M. T., Cofrades, S., & Carballo, J. (2003). Physicochemical and sensory characteristics of restructured beef steak with added walnuts. *Meat Science* 65, 1391–1397.

Kerry, J. K., O'Donnell, A., Brown, H., Kerry, J. P., & Buckley, D. J. (1999). Optimization of transglutaminase as a cold set binder in low-salt beef and poultry comminuted meat products using response surface methodology. In *Proceedings 45th International Congress of Meat Science and Technology* (pp. 140–141). 1–6 August, Yokohama, Japan.

Kilic, B. (2003). Effect of microbial transglutaminase and sodium caseinate on quality of chicken döner kebab. *Meat Science*, *63*(3), 417–421.

Kuraishi, C., Sakamoto, J., & Soeda, T. (1996). The usefulness of transglutaminase for food processing. In G. R. Takeoka, R. Teranishi, P. J. Williams, & A. Kobayashi (Eds.), *Biotechnology for improved food and flavors* (pp. 29–38). Washington DC: ACS (ACS Symposium Series 637).

Kuraishi, C., Sakamoto, J., Yamazani, K., Susa, Y., Kuhara, C., & Soeda, T. (1997). Production of restructured meat using microbial transglutaminase without salt or cooking. *Journal of Food Science*, *62*(3), 488–490, 515.

López-Caballero, M. E., Carballo, J., & Jiménez Colmenero, F. (1999). Microbiological changes in pressurized, prepackaged sliced cooked ham. *Journal of Food Protection*, *62*(12), 1411–1415.

Nielsen, G. S., Petersen, B. R., & Moller, A. J. (1995). Impact of salt, phosphate and temperature on the effect of a transglutaminase (FXIIIa) on the texture of restructured meat. *Meat Science*, *41*(3), 293–299.

O'Kennedy, B. (2000). Use of novel dairy ingredients in processed meats. In *End of the project report 1999: DPRC No. 15*. Teagast, Dublin, Ireland: Dairy Products Research Centre.

Penfield, M. P., Costello, C. A., McNeil, M. A., & Riemann, M. J. (1988). Effects of fat level and cooking methods on physical and sensory characteristics of restructured beef steaks. *Journal of Food Quality*, *11*(5), 349–356.

Pietrasik, Z. (2003). Binding and textural properties of beef gels processed with κ-carrageenan, egg albumin and microbial transglutaminase. *Meat Science*, *63*(3), 317–324.

Pietrasik, Z., & Jarmoluk, A. (2003). Effect of sodium caseinate and κ-carrageenan on binding and textural properties of pork muscle gels enhanced by microbial transglutaminase addition. *Food Research International*, *36*(3), 285–294.

Pietrasik, Z., & Li-Chan, E. C. Y. (2002). Response surface methodology study of the effect of salt, microbial transglutaminase and heating temperature on pork batter gel properties. *Food Research International*, *35*(4), 387–396.

Rocha-Garza, A. E., & Zayas, J. F. (1996). Quality of broiled beef patties supplemented with wheat germ protein flour. *Journal of Food Science*, *61*(2), 418–421.

Ruiz-Carrascal, J., & Regenstein, J. (2002). Emulsion stability and water uptake ability of chicken breast muscle proteins as affected by microbial transglutaminase. *Journal of Food Science*, *67*(2), 734–739.

Sabaté, J. (1993). Does nut consumption protect against ischaemic heart disease? *European Journal of Clinical Nutrition*, *47*(1), S71–S75.

Saleh, N. T., & Ahmed, Z. S. (1998). Impact of natural sources rich in provitamin A on cooking characteristics, colour, texture and sensory attributes of beef patties. *Meat Science*, *50*(3), 285–293.

Shao, C. H., Avens, J. S., Schmidt, G. R., & Maga, J. A. (1999). Functional, sensory and microbiological properties of restructured beef and emu steak. *Journal of Food Science*, *64*(6), 1052–1054.

Sheard, P. R., Nute, G. R., & Chappell, A. G. (1998). The effect of cooking on the chemical composition of meat products with special reference to fat loss. *Meat Science*, *49*(2), 175–191.

Trout, G. R., Chen, C. M., & Dale, S. (1990). Effect of calcium and carbonate and sodium alginate on the textural characteristics, color and color stability of restructured pork chops. *Journal of Food Science*, *55*(1), 38–42.

Tsai, S. J., Unklesbay, N., Unklesbay, K., & Clarke, A. (1998). Textural properties of restructured beef products with five binders at four isothermal temperatures. *Journal of Food Quality*, *21*(5), 397–410.

Tseng, T. F., Liu, D. D., & Chen, M. T. (2000). Evaluation of transglutaminase on the quality of low-salt chicken meat-balls. *Meat Science*, *55*(4), 427–431.

Wijngaards, G., & Paardekooper, E. J. C. (1988). Preparation of a composite meat product by means of enzymatically formed protein gel. In B. Krols, P. S. van Roon, & J. H. Houben (Eds.), *Trends in modern meat technology 2* (pp. 125–129). Wageningen, Netherlands: Pudoc.