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Characteristics of meat batters with added native and preheated defatted walnut

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Abstract

Effects of incorporation of native and preheated defatted walnut on the physicochemical, emulsifying and rheological properties of meat batters, as affected by final heating temperature were investigated. Replacing meat protein with native defatted walnut in meat product formulations reduced ($P < 0.05$) gel strength and emulsifying properties and hence the firmness and stability of meat batters but enhanced water- and fat-binding properties and hence the yield of a processed meat product. However, incorporation of preheated defatted walnut, in addition to improving ($P < 0.05$) water- and fat-binding properties during thermal treatment, improved the gelling ability of myofibrillar proteins, probably because the preheating of the defatted walnut promoted interactions between walnut proteins and muscle proteins.
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1. Introduction

Increasing consumer demand for healthier meat products has drawn further attention to the use of non-meat ingredients as potential sources of bioactive compounds. Numerous non-meat proteins from plant sources have been used as meat extenders and also as functional ingredients in muscle foods (Jiménez-Colmenero, Reig, & Toldrá, 2006). One of these is walnut, which has recently been used in the manufacture of meat products because of the potential positive health benefits that it confers (Serrano et al., 2005). Observational epidemiological studies show an inverse relationship between frequency of walnut consumption and risk of coronary heart disease (CHD) (Albert, Gaziano, Willett, & Manson, 2002; Fraser, Sabaté, Beeson, & Strahan, 1992). The FDA recently authorized a qualified health claim indicating that eating 42.5 g per day of walnuts, as part of a low-saturated-fat and low-cholesterol diet and not resulting in increased caloric intake, may reduce the risk of CHD (FDA, 2004). This effect has been associated with the peculiar blend of nutrients and phytochemical compounds found in walnuts: high-biological-value proteins (low lysine/arginine ratio), vegetable fibre, monounsaturated (oleic) and polyunsaturated (linoleic and α-linolenic) fatty acids and micronutrients such as folic acid, magnesium, liposoluble vitamins (especially γ-tocopherol) and other antioxidants (phytosterols and polyphenols). Moreover, the use of walnut as a non-meat ingredient would mean that it is possible to revalue those parts of the walnut, which because of their smallness or shape are not suitable for commercialisation.

Meat products (restructured beef steak and sausage) with added walnut have been formulated to produce products with acceptable physicochemical and sensory properties (Cofrades et al., 2004; Jiménez Colmenero et al., 2003). These reformulated meat products could be considered potential functional foods in that they incorporate biologically active components that have the potential to produce functional effects (Serrano et al., 2005).

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The protein content of walnut is around 15%, composed essentially of albumin (6.8%), globulin (17.6%), prolamin (5.3%) and glutelin (70.1%) (Sze-Tao & Sathe, 2000). Proteins of this kind are found in numerous food ingredients commonly added to processed meats to enhance product texture, fat- and water-binding properties. In comminuted muscle foods, the effect of this non-meat ingredient will depend to a large extent on how it interacts with muscle proteins. Interactions of myofibrillar and plant proteins (globulins, gluten) have been reported in muscle foods (Feng & Xiong, 2002; Shiga, Kami, & Fujii, 1988). Meat products are usually cooked to a final internal temperature of 68–73 °C; muscle proteins heated to these temperatures are essentially fully denatured, allowing the exposed reactive groups to impart desirable properties to processed meat. However, plant proteins generally require higher temperatures than do muscle proteins to unfold (Feng & Xiong, 2002). It has been reported that the high denaturation temperature of some proteins of this kind prevents the protein from undergoing sufficient structural change under the kind of thermal treatment normally applied to meat products, thereby limiting its interaction with the proteins of muscle systems (Feng & Xiong, 2002).

Preheating of soy and gluten proteins, before adding to the product formulation, has been reported to favour their ability to interact with the muscle proteins (Feng & Xiong, 2002). The mechanics of native and preheated soy protein action in meat are not fully understood (Feng & Xiong, 2002), but various types of interactions between plant protein fractions (7S globulins, dissociated 11S) and myofibrillar proteins have been reported (Feng & Xiong, 2002; King, 1977; Peng & Nielsen, 1986).

No data have been reported regarding possible interactions between walnut and muscle proteins and their effects on physicochemical properties of processed meats. The object of this work was to evaluate how the incorporation of native and preheated defatted walnut to meat batters affected formation of the protein gel/emulsion network. The study was based on an analysis of walnut and muscle proteins (salt-soluble protein) with respect to different meat batter physicochemical properties (gelling, water- and fat-binding properties, emulsifying capacity and dynamic rheological changes).

2. Materials and methods

2.1. Meat batter preparation

Select beef top rounds (15 kg) were trimmed of fat and connective tissue, cut into strips (approximately 5 × 4 × 20 cm) and passed once through a grinder (Mainca, Granollers, Spain) with 0.6 cm orifice plate diameter. Lots of approximately 0.5 kg, were vacuum-packed, frozen to −20 °C and stored until used. Walnut flour (supplied by Bernardo Josa, Carlet, Spain) was defatted according to Sze-Tao and Sathe (2000) and stored at −20 °C for further use. Half of the defatted walnut flour was placed on a glass tray, heated at 140 °C for 2 h and stored at 3 °C prior to use.

For the preparation of meat batters, meat packages were thawed (approximately 18 h at 3 ± 2 °C). Three different meat batters with a target final protein level of 15% (sum of meat and walnut protein) were prepared (C, no walnut added; MWn, native defatted walnut added; MWh, preheated defatted walnut added) (Table 1). The procedure was as follows: raw meat material was homogenized for 1 min in a chilled cutter (Stephan Universal Machine UM5, Stephan u. Sóhne GmbH & Co., Hameln, Germany). Sodium chloride (2.5%) (Panreac Quimica, S.A. Barcelona, Spain), dissolved in chilled water, was added to the meat and homogenized for 1 min. Defatted walnut flour (native or preheated) was sprinkled on top of the mixture and the whole homogenized again for 1 min. Finally, the whole meat batter was homogenized under vacuum conditions for 1 min. Mixing time was standardized at approximately 5 min. The final batter temperature was below 12 °C in all cases.

Portions of each meat batter (approximately 35 g) were placed in plastic containers (diameter 3.4 cm, height 7 cm), hermetically sealed and stored (3 °C) for 2 h. The containers with each of the three samples (C, MWn and MWh) were selected at random and half was heated to an internal temperature of 40, 50, 60 and 70 °C in a controlled water bath. The internal temperature was measured using thermocouples connected to a temperature recorder (Yokogawa Hokushin Electric YEW, Mod. 3087, Tokyo, Japan). Immediately after heating, the containers were cooled in ice water (30 min). Both unheated (half containers) and heated samples were stored in a chilling room at 3 °C prior to analysis.

2.2. Chemical analysis

Moisture and ash contents of meat and meat batters were determined (AOAC, 2000) in triplicate. Protein content was measured in quadruplicate by a Nitrogen Determinator LECO FP-2000 (Leco Corporation, St. Joseph, MI). Fat content was determined in duplicate according to Bligh and Dyer (1959).

2.3. Salt-soluble protein and gel electrophoresis

Ten gram of each sample were homogenized in an Omni-Mixer (ES Homogenizer, OMNI International Inc., 6530 Commerce Ct. Gainsville, VA) for 90 s at 2–4 °C with

Table 1
Formulation of different meat batters

Samples	Beef (g)	Salt (g)	Walnut (g)	Water (g)	Total (g)
C	456.47	17.5	0	226.03	700
MWn	365.19	17.5	46.62	270.69	700
MWh	365.19	17.5	46.62	270.69	700

C = no walnut added; MWn = native defatted walnut added; MWh = preheated deffated walnut added.

50 ml of 0.6 M NaCl (20 mM phosphate, pH 7.0) solution. The slurry was centrifuged (Beckman J2MC CA, USA) for 30 min at 27,200*g* and 4 °C. Protein solubility in the supernatant of the different samples was determined according to Lowry, Rosebrough, Farr, and Randall (1951) using specific standard curves with bovine serum albumin. The results were expressed as the percentage of solubilized protein with respect to total protein content of the meat batters (AOAC, 2000). This fraction was denominated salt-soluble protein and it is considered to be essentially native protein (Jiang, San, & Japit, 1989).

Salt-soluble protein of raw meat batters and batters heated to 70 °C (C, MWn and MWh) were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Protein fractions extracted with 0.6 M NaCl from the defatted native (Wn) and preheated walnut (Wh) were also analysed. The samples were treated according to the method of Hames (1985) (2% SDS, 5% β-mercaptoethanol, and 0.002% bromophenol blue) and then heated for 5 min in a boiling water bath. Samples were electrophoresed on 12.5% acrylamide gels, following the procedure of Laemmli (1970). Electrophoresis was performed in a 7 × 8 × 0.75 cm PROTEAN II dual slab cell (Bio-Rad, Richmond, CA) at a constant 20 A, according to the manufacturer's instructions. Samples were diluted to a concentration of 4 mg/ml. Approximately 10 μl of protein were loaded into each well. The gel was stained with Coomassie Brilliant Blue R-250. The molecular weight of the main proteins was calculated by comparing their mobility with a standard high molecular weight protein mix (Pharmacia LKB Biotechnology, Uppsala, Sweden).

2.4. Dynamic rheological measurement

Rheological changes in unheated samples (C, MWn and MWh) during thermal gelation were studied using a Bohlin CSR-10 Rheometer (Bohlin Instruments Inc., Cranbury, NJ, USA) operating in small-amplitude oscillatory mode (Cofrades, Ayo, Serrano, Carballo, & Jiménez Colmenero, 2006). After equilibration at room temperature (20 °C), thermal gelation was induced by heating samples from 20 to 90 °C at 1 °C/min, using a Bohlin temperature unit. Samples were sheared at a fixed frequency of 1.0 Hz with a strain of 0.02. The gap between the plates was set at 1 mm. The sample perimeter was covered with a thin layer of silicone oil to prevent dehydration. Changes in the storage modulus (G', i.e. rigidity due to elastic response of the material) were monitored throughout the gelling process. The rheograms presented are means of the two different measurements (replications).

2.5. Penetration force

This parameter was evaluated using a penetration test in which a stainless-steel rod (diameter 5 mm) was attached to a 100 N cell connected to the crosshead of an Instron model 4501 Universal texturometer (Instron Engineering Corporation, Canton, MA). The rod penetrated 10 mm into the gel at a crosshead speed of 20 mm/min. The force–penetration curves were analyzed using a Hewlett-Packard Vectra ES/12 computer. The penetration force (PF) (N), was defined as the load required for the sample to rupture. When protein structures did not break in the maximum travel of the plunger (10 mm), the PF was taken as the load enclosed by force–distance curve at that distance. Determinations were carried out five times.

2.6. Water- and fat-binding properties

The samples heated to 60 and 70 °C were used to determine water- and fat-binding properties. After heating, the containers were opened and left to stand upside down (for 30 min) to release the exudate. Total loss (TL) was expressed as % of initial sample weight. Water loss (WL) was determined as % weight loss after heating the total released fluid (TL) for 16 h on a stove at 100 °C. Fat loss (FL) was calculated as the difference between TL and WL. WL and FL were expressed as % of total loss. Determinations were carried out in triplicate.

2.7. Emulsifying capacity

Emulsifying capacity of raw meat batters was performed according to Jiménez Colmenero and García Matamoros (1981). The results were expressed as g of oil/g of sample.

2.8. Statistical analysis

Data were analysed using using Statgraphics plus 2.1 (STSC Inc., Rockville, MD) for one-way and two-way ANOVA, depending on the target parameter. Least squares differences were used for comparison of mean values among treatments and the Tukey HSD test was used to identify significant differences ($P < 0.05$) among main effects of presence of walnut and heating temperatures.

3. Results and discussion

3.1. Proximate composition

Protein contents of raw meat batters were close to the target level of 15% (Table 2). The different meat batters

Table 2
Proximate analysis (%) of raw meat batters

Samples	Moisture	Protein	Ash	Fat
C	79.73[a]	15.2[a]	3.59[a]	2.23[a]
MWn	77.41[a]	15.5[a]	3.38[a]	3.90[b]
MWh	78.41[a]	16.0[b]	3.33[a]	3.89[b]
SEM	0.85	0.09	0.29	0.13

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

did not differ significantly in moisture, protein or ash contents. The incorporation of defatted walnut flour produced an increase ($P < 0.05$) in fat content as the fat content of the defatted walnut (12%) was higher than that of the meat raw material (2%).

3.2. Salt-soluble protein and gel electrophoresis

3.2.1. Protein solubility

Table 3 shows the concentrations of salt-soluble proteins (SSP) of different meat batters. In the raw meat batters, SSP was higher ($P < 0.05$) in the sample with added native walnut (MWn) than in the control or WMh samples.

All samples showed a significant decrease in salt-soluble protein with increasing heating temperature (Table 3). MWn and MWh registered the smallest decrease (about 60%) in SSP concentration when batter was heated to 70 °C, while SSP concentration in C dropped by approximately 80%. Similar behaviours have been reported in various meat batters with low and high fat contents (Cofrades & Jiménez-Colmenero, 1996). It has been established that, during heating, the protein chain unfolds as the temperature rises, and muscle proteins heated to 70 °C are essentially fully denatured. The main reason why salt-soluble protein content was higher in meat batters with added walnut (MWn and MWh) heated to 60 °C (~15–16%, respectively) and 70 °C (~12–10%, respectively) than in the control (~10% at 60 °C and ~5% at 70 °C), even although the total protein contents were similar, may be that walnut proteins are more thermostable than are muscle proteins (Feng & Xiong, 2002; Nagano, Fukuda, & Akasaka, 1996). The effects of walnut preheating were not clearly apparent in the SSP since no differences ($P > 0.05$) were observed between MWn and MWh (Table 3).

Table 3
Salt-soluble protein content (SSP) (% with respect to the total protein content of the meat batters) affected by heating temperature and formulation

Temperature	C	MWn	MWh
Raw	28.2^{a}_{1}	31.5^{b}_{1}	26.7^{a}_{1}
40 °C	23.3^{a}_{2}	27.4^{b}_{2}	19.9^{c}_{2}
50 °C	20.5^{a}_{2}	21.5^{a}_{3}	19.0^{a}_{2}
60 °C	10.1^{a}_{3}	15.8^{b}_{4}	16.2^{b}_{2}
70 °C	5.47^{a}_{4}	12.6^{b}_{5}	10.2^{b}_{3}
SEM	0.77	0.77	0.77

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

3.2.2. Gel electrophoresis

The electrophoretic patterns of SSP in control (raw and heated to 70 °C) and defatted native and preheated walnut (Wn and Wh) are shown in Fig. 1. SDS-PAGE patterns of SSP in raw meat batter and batter heated to 70 °C with native (MWn) and preheated defatted walnut (MWh) are shown in Fig. 2.

SSP of raw meat batter without walnut (C) shows the typical profile of muscle proteins (Fig. 1a). There are two main bands, myosin heavy chain (MHC) and actin (A), and some less intense bands compatible with C-protein, α-actinin, tropomyosin (TM), troponins (TN) and myosin light chains (MLC) (Margossian & Lowey, 1982). It is well known that, as the temperature increases, important structural changes take place in the myosin, producing protein denaturation (Wright, Leach, & Wilding, 1977). In addi-

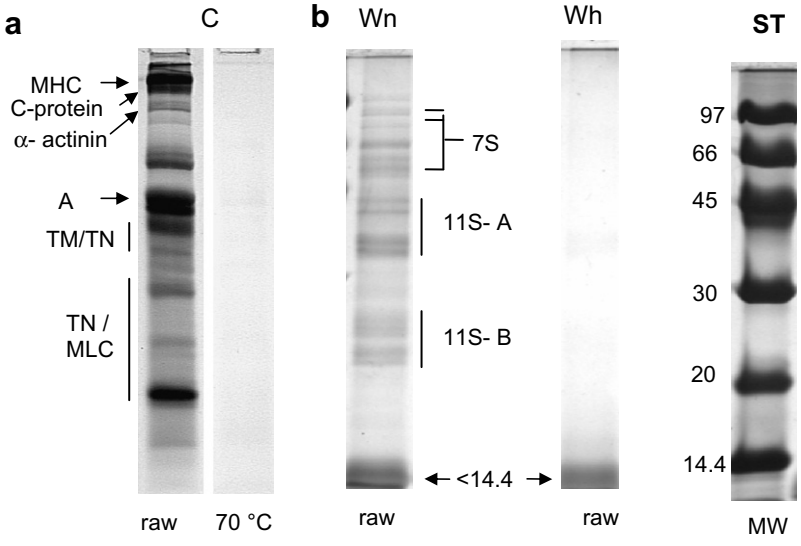

Fig. 1. SDS-PAGE patterns of salt-soluble protein obtained from (a) meat batter (without added walnut, C), raw and after heating to 70 °C, and (b) native defatted walnut (Wn) and preheated defatted walnut (Wh). ST = standard molecular weight (in kDa). MHC = myosin heavy chain; A = actin; TM/TN = tropomyosin/troponin-T; TN/MLC = troponin-I, C/myosin light chains. 7S = 7S component; 11S-A = acidic polypeptide of 11S component; 11S-B = basic polypeptide of 11S component of walnut proteins.

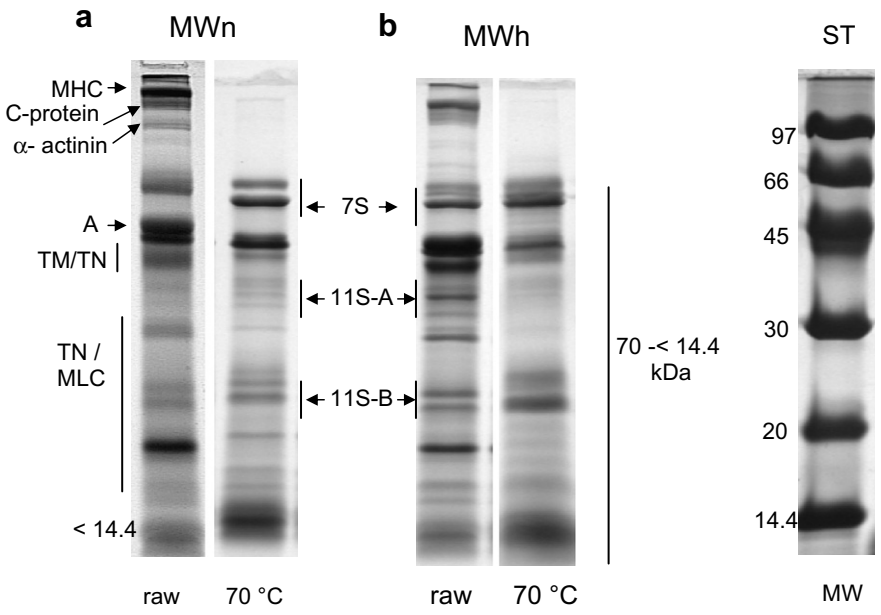

Fig. 2. SDS-PAGE patterns of salt-soluble protein obtained from (a) meat batter with native walnut added (MWn), raw and after heating to 70 °C, and (b) meat batter with preheated walnut added (MWh), raw and after heating to 70 °C. ST = standard molecular weight. MHC = myosin heavy chain; A = actin; TM/TT = tropomyosin/troponin-T; TN/MLC = troponin-I, C/myosin light chains. 7S = 7S component; 11S-A = acidic polypeptide of 11S component; 11S-B = basic polypeptide of 11S component of walnut proteins.

tion to an effect on protein solubility (Table 3), this implies that, at 70 °C, there are no detectable bands corresponding to myofibrillar muscle protein.

SDS-PAGE patterns of SSP of native and preheated walnut are shown in Fig. 1b. In the native walnut (Wn), numerous polypeptide bands are visible, with molecular weights ranging from 112 kDa to <14.4 kDa. They are made up largely of albumin and globulin components (Mc Cord, Smyth, & O'Neill, 1998; Sze-Tao & Sathe, 2000). The doublet from ~21 to ~23 kDa and from ~32 to ~34 kDa is comparable to the acid and basic polypeptides of 11S-like proteins, and the range of polypeptides with molecular weights between ~68 and ~56 kDa could be compatible with α′,α- and β-conglycinin (7S) protein (Feng & Xiong, 2002; Morales-Arellano, Chagolla-Lopez, Paredes-Lopez, & Barba de la Rosa, 2001). In the SSP from preheated defatted walnut (Wh), practically all the polypeptide bands have disappeared except for the ones at MW < 14.4 kDa (Fig. 1b), which are compatible with albumins (Sze-Tao & Sathe, 2000).

SDS-PAGE patterns of salt-soluble proteins in MWn and MWh samples are shown in Fig. 2. Compared with the control (C, without walnut), the sample with defatted native walnut incorporated (MWn) presents notable changes in the electrophoretic profile (Figs. 1a and 2a). In the samples heated to 70 °C, unlike the control (Fig. 1a), the MWn sample shows an electrophoretic profile with numerous polypeptide bands at molecular weights ranging from 66 to 12 kDa (Fig. 2a). Meat batters containing preheated defatted walnut (MWh) presented similar results (Fig. 2b) but, in the samples heated to 70 °C, the number of bands was smaller than in MWn sample (Fig. 2a). Similarly, in the MWh sample heated to 70 °C (Fig. 2b), the actin band was less intense and the bands corresponding to peptides of MW < 14.4 kDa were more intense than in the sample with native walnut (MWn) (Fig. 2a). This is so despite the above-noted differences in the electrophoretic profiles of native and preheated walnut samples (Fig. 1b).

It seems clear, from the electrophoretic bands of the SSP, that samples containing walnut (MWn and MWh) and heated to 70 °C contain appreciable amounts of myofibrillar proteins, as well as walnut proteins (Fig. 2a and b). This is clearly the consequence of less denaturation of muscle proteins during the heating process. It has been suggested that plant proteins prevent the muscle protein from undergoing sufficient structural changes under the kind of thermal treatment commonly applied to meat products, thereby limiting its interaction with the proteins of muscle systems (Feng & Xiong, 2002). In the present study, the protective effect during thermal denaturation of myofibrillar protein was evident with both native and preheated walnut proteins; this is possibly related to interactions between animal and plant proteins, producing some larger soluble molecular structures (Fig. 2). The intense band at MW < 14 kDa, appearing in the profile of the meat batters formulated with native walnut (Fig. 2a) and walnut preheated at 70 °C (Fig. 2b), suggests that there were few protein–protein interactions and hence these had little implication in the formation of molecular structures produced in the process of thermal gelling. The augmented presence of protein observed at 70 °C (Fig. 2) is consistent with the higher proportion of SSP in MWn and MWh than in C at that temperature (Table 3).

We have found no comparable studies on the effects of incorporating walnut proteins in meat systems, but the results of this research are consistent with the findings of Feng and Xiong (2002) in a study of myofibrillar protein mixed with native and preheated soy protein isolate. These authors suggested that the earlier onset of myosin disappearance probably resulted from the interaction between β-conglycinin and myosin. Peng and Nielsen (1986) reported interaction between β-conglycinin and myosin heavy chain of soy 7S during heating from 50 to 100 °C.

3.3. Dynamic rheological testing

Fig. 3 shows the storage modulus (G') as a function of temperature for different meat batters, C, MWn and MWh. The main rheological changes during heating of control meat batter (C) (no walnut added) occurred at above ~50 to ~60 °C, where there was a sharp increase in G', indicating the formation of a stiff elastic matrix structure typical of heat-induced protein gels. This rheological pattern was typical of myofibrillar protein gels with added salt and has been well documented by other authors from avian and bovine skeletal muscles (Egelandsdal, Fretheim, & Samejima, 1986; Fretheim, Samejima, & Egelandsdal, 1986; Wang, Smith, & Steffe, 1990; Xiong & Blanchard, 1993). The storage modulus of defatted native and preheated walnut underwent no substantial changes during heating (data not shown), which would indicate a very scant incidence of conformational changes in the protein associations which lead to the formation of gel structures. As far as the authors know, no data have been reported on dynamic rheological testing of walnut proteins, but similar results have been reported by various authors in soy protein isolates (Feng & Xiong, 2002; Mc Cord et al., 1998; Nagano et al., 1996).

Addition of defatted walnut produced some differences in the rheological behaviour of samples during heating as compared with the control (Fig. 3). From 30 °C up to ~58 °C, addition of walnut (native or preheated) produced higher G' values than those in control meat batters. The shifts caused by preheated walnut were larger than those caused by native walnut (Fig. 3). Since hydrophobic interactions are important in stabilizing the unheated batter (Gordon & Barbut, 1992), relatively low temperatures could favour such interactions between meat protein and walnut protein in preheated walnut. At higher temperatures (>60 °C), meat batter formulated with preheated walnut (MWh) also presented higher values of G', whereas, the samples prepared with native walnut (MWn) behaved the same as did the control (C), with lower values of G' at above 70 °C (Fig. 3). These rheological patterns may be due to the occurrence of more preheated walnut protein/muscle protein interactions, which are accompanied by enhanced gelling ability, as reported in the case of soy protein (Feng & Xiong, 2002). The presence of native walnut in meat batter (MWn) appears to produce the opposite effect at higher temperatures (>70 °C). Such a diminishing of gelling properties is consistent with a report by Ramírez-Suarez and Xiong (2003) that non-modified soy proteins had a detrimental effect on muscle protein gelation, probably by hindering the type of protein–protein interaction responsible for high elasticity. The results for SSP appear to corroborate the assumption that protein aggregation was augmented when preheated walnut was added to meat batter (Table 3). These effects were consistent with the electrophoretic analysis where, at 70 °C, the intensity of the protein bands for walnut was greater in the MWh than in the MWn sample (Fig. 2). This behaviour could indicate that proteins from preheated walnut were more implicated in aggregation than were proteins from native walnut.

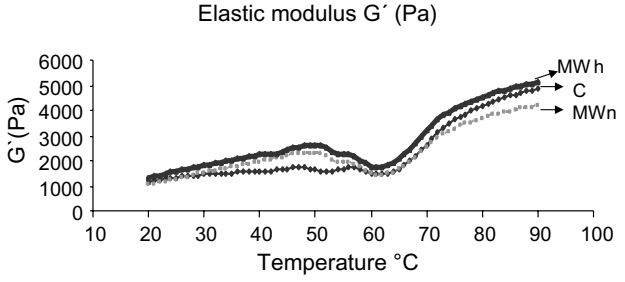

Fig. 3. Elastic modulus (G') as a function of heating temperature of different meat batters. C, no walnut added; MWn, meat batter with native defatted walnut added; MWh, meat batter with preheated defatted walnut added.

3.4. Penetration force

The gelling properties of the different samples (C, MWn and MWh) were assessed by measuring the penetration force (PF) of the gels that formed at different temperatures (Table 4). In all cases (regardless of walnut addition), increased temperature was associated with greater gel strength, with maximum PF values registered at 70 °C. This behaviour is due to a dynamic process which involves protein unfolding and aggregation prior to the formation of stable, stiff, elastic matrix structures typical of heat-induced protein gels.

The incorporation of walnut affects that process and hence the properties of heated meat batters. The changes occurring in gel formation depend both on the state of the added walnut (native or preheated) and on the final gelling temperature. In that respect, the consequences of

Table 4
Penetration force (N) of meat batters heated to various temperatures

Penetration force (N)	40 °C	50 °C	60 °C	70 °C
C	0.30^{a}_{1}	1.05^{b}_{1}	3.70^{c}_{1}	4.73^{d}_{1}
MWn	0.35^{a}_{1}	0.69^{b}_{2}	1.80^{c}_{2}	2.60^{d}_{2}
MWh	0.51^{a}_{1}	1.01^{b}_{1}	4.39^{c}_{3}	4.76^{d}_{1}
SEM	0.75	0.75	0.75	0.75

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

walnut incorporation were more pronounced at high temperatures. While at 40 °C all samples presented the same PF values ($P > 0.05$), as the final gelling temperature increased, considerable differences emerged between the heated meat batters. As compared to the control (C), the addition of native walnut reduced ($P < 0.05$) penetration force, whereas, with the exception of the sample heated to 60 °C, addition of preheated walnut (MWh) produced gels with similar ($P > 0.05$) PF levels (Table 4).

The differences observed between control meat batter (C) and meat batter with native walnut added (MWn) in the PF behaviour model, as affected by gelling temperature, are consistent with the results of dynamic rheological testing (Fig. 3). The presence of native walnut protein can limit the formation of the kind of molecular associations implicated in protein gel network formation, producing structures with lower resistance to penetration. Some authors have noted that addition of non-meat protein (whey protein isolate, soy protein isolate) to salt-soluble protein, in fish surimi or turkey breast, reduced gel strength as compared to those of pure muscle protein gels (Feng & Xiong, 2002; Lanier, 1991; Mc Cord et al., 1998). The fact that MWn samples exhibited less PF than did the controls (Table 4) is consistent with the given levels of protein solubility (Table 3). Some authors have suggested the possibility that native walnut protein interferes with thermal gelling processes in meat muscle products (Cofrades et al., 2004; Jiménez Colmenero et al., 2003). This effect was attributed to a combination of several factors connected with increased fat and reduced water, affecting the characteristics of the matrix, to the diluting effect of walnut in meat protein systems, and to possible interferences in meat protein gelling processes, all of which can, to some extent, limit binding between meat pieces.

Unlike the case of variation of G', the presence of preheated walnut did not cause the formation of stronger heat-induced structures in the mixed protein system (MWh), as reflected by PF values (Table 4). Discrepancies between the two measuring procedures have been reported in the literature, arising from the different natures of the measurements and from the fact that the measuring temperature differs in the two procedures (Carballo, Cofrades, Fernández Martín, & Jiménez-Colmenero, 2001).

3.5. Water- and fat-binding properties

Water- and fat-binding properties (TL, WL and FL) of different meat batters heated to 60 and 70 °C are presented in Table 5. Total losses (TL) were higher in the samples heated to 70 °C than to 60 °C, a pattern widely reported by various authors (Carballo, Fernández, Barreto, Solas, & Jiménez-Colmenero, 1996). In the samples containing walnut (native or preheated) and heated to 70 °C, the binding parameters (TL, WL and FL) were lower ($P < 0.05$) than in the control (Table 5). This indicates that incorporation of walnut, regardless of its state (native or preheated), improves water-holding capacity during cooking. Similar results have been reported in restructured steak and sausage with walnut added (Cofrades et al., 2004; Jiménez Colmenero et al., 2003). The differences in the rheological characteristics of gels formed with native and preheated walnut (Fig. 3 and Table 4) had no clear effect on water- and fat-binding properties (Table 5) as other authors have reported (Camou & Sebranek, 1991).

Table 5
Water- and fat-binding properties

	TL	WL	FL
Samples 60 °C			
C	0.87[a]	91.46[a]	8.54[a]
MWn	0.15[b]	89.75[a]	10.24[a]
MWh	0.14[b]	74.90[b]	25.10[b]
SEM	0.18	1.98	1.98
Samples 70 °C			
C	12.27[a]	93.11[a]	6.89[a]
MWn	6.61[b]	88.04[b]	11.96[b]
MWh	4.15[b]	88.01[b]	11.99[b]
SEM	0.57	0.17	0.17

Total (TL, % of sample weight), water (WL, % of TL) and fat (FL, % of TL) losses.
For sample denomination see Table 1. Different letters in the same column indicate significant differences ($P < 0.05$). SEM = standard error of the mean.

3.6. Emulsifying capacity

Table 6 shows the emulsifying capacity (EC) of the different raw meat batters (C, MWn and MWh). The added walnut produced a reduction ($P < 0.05$) in emulsifying capacity, which was more pronounced in the MWh sample. Whereas, all the protein in the control was from meat, MWn and MWh samples contained mixed protein systems (from muscle and walnut proteins). The greater proportion of the muscle protein in the control sample (C) would account for its greater ability (g oil/g sample) to encapsulate fat (Table 6). It is well known that myofibrillar proteins present better emulsifying properties than do globular proteins (Borderías, Jiménez Colmenero, & Tejada, 1985). The fact that EC was lower ($P < 0.05$) in the MWh than in the MWn sample may be a consequence both of the higher level of SSP in MWn (Table 3, Fig. 2a) and of differences in the interaction between muscle and walnut (native and heated) proteins. Thermally denatured walnut

Table 6
Emulsifying capacity (g oil/g sample and g oil/mg soluble protein) of meat batters

Samples	g oil/g sample
C	29.31[a]
MWn	26.44[b]
MWh	24.64[c]
SEM	0.28

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

proteins can reduce the degree of unfolding of polypeptides of muscle protein that occurs during the shearing involved in emulsification. This effect is different from the effect on gelling ability properties (Table 4).

4. Conclusions

Our results indicate that replacing meat protein with native defatted walnut in meat product formulations reduced gel strength and emulsifying capacity but enhanced water- and fat-binding properties and hence the yield of a processed meat product. However, the incorporation of preheated defatted walnut additionally improves the thermal gelling ability of myofibrillar proteins, probably because the preheating of the defatted walnut promotes walnut/muscle protein interactions. This suggests that defatted preheated walnut may have a protective effect on meat proteins. Therefore, further studies are needed to examine the relationship between the process of walnut protein denaturation (preheating time and temperature) and the functional properties of mixtures with meat proteins. It will also be necessary to determine which walnut proteins are most involved and the exact mechanism of interaction between meat proteins and walnut proteins.

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