HEALTHIER LIPID COMBINATION OIL-IN-WATER EMULSIONS PREPARED WITH VARIOUS PROTEIN SYSTEMS. AN APPROACH FOR THE DEVELOPMENT OF FUNCTIONAL MEAT PRODUCTS

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List of Abbreviations: MS meat slurry, **MTG** microbial transglutaminase, **SC** sodium caseinate, **SEM** Scanning electron microscopy, **SFA** saturated fatty acids, **SPI** soy protein isolate.

Abstract

Five protein-stabilized oil-in-water emulsions were prepared using sodium caseinate (O/SC), soy protein isolate (O/SPI), sodium caseinate and microbial transglutaminase (O/SC þ MTG), sodium caseinate, microbial transglutaminase and meat slurry (O/SC þ MTG þ MS) and SPI, sodium caseinate and microbial transglutaminase (O/IPS þ SC þ MTG); their composition (proximate analysis and fatty acid profile) and physicochemical characteristics were examined. The lipid phase was a combination of healthy fatty acids from olive, linseed and fish oils, containing low proportions (15%) of saturated fatty acids (SFA) and high proportions of monounsaturated fatty acids (MUFA, 47%) and polyunsaturated fatty acids (PUFA, 36%), with a PUFA/SFA ratio >2, and a n-6/n-3 PUFA ratio of 0.4. All the oil-inwater emulsions showed high thermal and creamy stability. Results of penetration test and dynamic rheological properties showed la existencia de different types of oil-in-water emulsion structures according to stabilizing system of emulsion. Those structures ranged from concentrate solution-like (stabilized only with SC) (gel strength 0.06 mJ) to gel-like (samples containing MTG) behaviours (gel strength ranged between 3.4 and 6.2 mJ). Morphological differences in the organization of the network structure were observed (by scanning electron microscopy) as functions of the protein system used to stabilize the oil-in-water emulsions

1 Introduction

Diet and nutrition are important factors in the promotion and maintenance of good health throughout a person's lifetime. Nutrition is coming to the fore as a major modifiable determinant of chronic disease, with scientific evidence increasingly supporting the view that alterations in diet have strong effects, both positive and negative, on health throughout life [1]. There is growing evidence that dietary fat may play a role in the prevention and treatment of a number of chronic disorders, particularly coronary heart disease. These findings have prompted the issue of dietary recommendations for optimal intake of total, saturated and unsaturated fatty acids, including the proportion between them [1-3]. Dietary fat intake should constitute between 15 % and 30 % of total diet energy, saturated fatty acids (SFA) no more than 10 %, polyunsaturated fatty acids (PUFA) around 6-10 % (n-6, 5-8 %; n-3, 1-2 %), monounsaturated fatty acids (MUFA) around 10-15 %, and less than 1 % should be from *trans* fatty acids. It is also recommended to limit cholesterol intake to 300 mg/day [1].

Like other food-related sectors, the meat industry is undergoing major transformations, driven among other things by changes in consumer demands. One of the main trends shaping developments in the consumption of meat derivatives is consumer interest in the possibilities of improving health through diet. Meat-based functional foods are seen as an opportunity to improve their "image" and address the needs of consumers, as well as to update nutrient dietary goals. Because of their importance, lipids are among the bioactive components (functional ingredients) that have received most attention, particularly (in quantitative and qualitative terms) with respect to the development of healthier meat products [4-7].

Reformulation of meat derivatives is one of the strategies that has been studied in order to develop healthier meat products (potential functional foods). Healthier lipid reformulation is generally based on replacement (to a greater or lesser extent) of the animal fat normally present in the product with another fat whose characteristics are more in line with health recommendations: i.e., with smaller proportions of SFA and larger proportions of MUFAs or PUFA (especially long chain n-3 PUFA), better n-6/n-3 PUFA and PUFA/SFA ratios, and if possible cholesterol-free. A variety of non-meat fats of plant (olive, linseed, canola, etc.) and marine (fish and algae) origin have been added to different meat products as partial substitutes for meat fats, mainly pork or beef [7].

Different technology options for animal fat replacement have been assayed for the development of healthier lipid meat products. Because of their physicochemical characteristics, pre-emulsions (oil-in-water emulsions) are a suitable technological option to stabilize the non-meat fats used for incorporation in meat derivates [7-9]. Pre-emulsions are made prior to meat product manufacture and are added to meat products as fat ingredients. Oil-in-water emulsion technology with a non-meat protein improves the system's fat binding ability, since the oils can be stabilized or immobilized in a protein matrix. This leaves more meat protein available to act in the system and reduces the chances of bulk oil physically separating from the structure of the meat product so that it remains stable throughout the range of environmental conditions that it is likely to encounter during processing, storage and consumption [10]. Besides being physically stable throughout a product's lifetime, oil-in-water emulsions constitute an excellent means of enhancing the oxidative stability of lipids in bulk oils, as additional protective measures such as antioxidants can be used to inhibit lipid oxidation. Also, oil-in-water emulsions are easier to disperse into water-based systems such as muscle foods [10].

A number of procedures have been reported for producing an oil-water emulsion for incorporation in meat derivatives [7]. The most commonly applied is one proposed by Hoogenkamp [11, 12], which has been used in numerous applications [13-16]. Generally, sodium caseinate (SC) has been used as an emulsifier in sausage-type products, whereas soy protein isolate (SPI) has been used in fermented products. Vegetable oils have been used as sources of MUFA and PUFA and marine oils as sources of eicosapentenoic (EPA) and docosahexaenoic (DHA) fatty acids to produce health-promoting fatty acid-enriched meat products [7]. The literature contains information on the characteristics of various different types of oil-in-water emulsions for food applications [17, 18], but their technological properties (stability, gelling ability, etc.) are considerably affected by variations in different factors, including the combination of ingredients such as oil and emulsifier. As far as the authors are aware, there are no references in the literature explaining the characteristics of the oil-in-water emulsions used for animal fat replacement, especially when healthier lipid combinations (of plant and marine origin) are used. This applies to lipid mixtures so designed that healthier meat products containing them as ingredients present better fatty acid profiles and high enough concentrations of specific beneficial fatty acids to make a serious contribution to recommended intake levels when the food is consumed in normal quantities.

One fundamental requirement of the design and reformulation of these products with a view to potential health benefits is to assure that lipid contents and profiles meet recommended nutritional goals. Paneras et al. [19] reported the formulation of frankfurters with vegetable oils (olive, cottonseed and soybean) following dietary guidelines for fatty acids.

Oil-in-water emulsions of this type are added to meat products as fat ingredients, and hence their composition and physicochemical characteristics affect different quality properties of the reformulated product [7]. A fuller understanding of their characteristics will therefore facilitate their use, help to elucidate their role in the protein matrix structure and help to improve the quality of meat-based food systems to which they are added. The aim of this experiment was to analyse the fatty acid composition and physicochemical characteristics of oil-in-water emulsions stabilized with various protein systems formulated using sodium caseinate, soy protein isolate, meat protein and microbial transglutaminase. The lipid phase was a combination of olive, linseed and fish oils, specially designed with fatty acids in suitable amounts and proportions for purposes of achieving healthier intake goals.

2 Materials and methods

2.1 Materials and reagents

Lipid sources were: olive oil (Carbonell Virgen Extra, SOS Cuétara SA, Madrid, Spain), linseed oil (Natursoy S.L., Alimentos Ecológicos, Castellterçol, Spain) and fish oil (Omevital 18/12 TG Gold) from Cognis GmbH, Illertissen, Germany, according to supplier information containing 160 mg EPA/g and 115 mg DHA/g plus a combination of tocopherols as antioxidants. The emulsion was made with: meat (mixture of *M. biceps femoris*, *M. semimembranosus*, *M. semitendinosus*, *M. gracilis* and *M. adductor*) from a local market, sodium caseinate (SC) 86.4% of protein content (Julio Criado Gómez SA, Alcorcón, Spain), soy protein isolate (SPI) 92.1 % of protein concentration (Vicoprot, TRADES S.A., Barcelona, Spain) and microbial transglutaminase (MTG) (ACTIVA WM, Ajinomoto Europe Sales GmbH, Hamburg, Germany). According to suplier information, the enzyme was in a mixture containing 1 % transglutaminase and 99 % maltodextrin, with a standard transglutaminase activity of approximately 100 units/g.

2.2 Preparation of oil-in-water emulsions

Five different types of oil-in-water emulsions were formulated (Table 1) and prepared using a homogenizer (Stephan Universal Machine UM5, Hameln, Germany) with a control bath at 5 ºC. The lipidic material, which was the same in all cases, consisted of a combination of olive, linseed and fish oils in respective proportions of 44.39, 37.87 and 17.74 %. This oil combination was designed to produce a healthier lipid formulation with a small proportion of SFA, large proportions of MUFA and PUFA (including long chain n-3 PUFA) and balanced n-6/n-3 PUFA and PUFA/SFA ratios in line with health recommendations. Although the lipid fatty acids profile as designed constitutes a healthier lipid formulation in itself, when mixed in different proportions with meat raw material (during meat product manufacture) it can help to obtain a meat product with improved fat content in line with dietary recommendations for optimal intake of total, saturated and unsaturated fatty acids, including the proportion between them [1-3].

The oil-in-water stabilized with sodium caseinate (O/SC) was prepared by mixing eight parts of water with one part of sodium caseinate for 2 min in the Stephan Universal Machine. The mixture was emulsified with 10 parts of the oil combination for another 3 min. The emulsion stabilized with soy protein isolate (O/SPI) was prepared by mixing eight parts of hot water (60–65 8C) with one part of SPI for 2 min in the Stephan Universal Machine. That mixture was then emulsified for a further 3 min with 10 parts of the oil combination. Both oil-in-water emulsions (O/SC and O/SPI) were prepared according to the procedure of Hoogenkamp [11, 12]. They have been used in the manufacture of numerous cooked and fermented meat products [7].

The oil-in-water stabilized with sodium caseinate and microbial transglutaminase (O/SC+MTG) was prepared in the same way as O/SC but including MTG (Table 1). A meat slurry (MS) was prepared by homogenizing (Omnimixer 2 min) 23.58 g of meat with 608 ml of water (2 °C) for use in preparation of the O/SC+MTG+MS emulsion. This sample was prepared by mixing eight parts of MS with one part of SC and MTG in the Stephan Universal Machine (Table 1) for 2 min. The mixture was emulsified for a further 3 min with 10 parts of the oil combination. The oil-in-water stabilized with soy protein isolate, sodium caseinate and

microbial transglutaminase (O/IPS+SC+MTG) was prepared in the same way as O/SPI but including SC and MTG (Table 1).

The temperature of the emulsions was less than 10 °C (Temperature Logger EBI-2T-211, ebro Electronic GmbH & Co.KG, Ingolstadt, Germany) at the end of the process. Each sample was stuffed into plastic tubes (length 11.5 cm, diameter 2.7 cm, weight approximately 56 g) and centrifuged (2500 g, 3 ºC, 5 min) (Multifuge 3L-R, Kendro Laboratory Products GmbH, Hanau, Germany). The oil-in-water emulsions were stored 24 h at 2 º± 2 ºC, before analysis. Each oil-in-water emulsion was prepared in duplicate.

2.3 Proximate analysis and pH

Sample moisture and ash contents were determined [20] in triplicate. Protein content was measured in triplicate with a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI, USA). Fat content was evaluated in triplicate according to Bligh and Dyer [21]. The pH was determined six times using an Orion Research 720A pH meter (Instrumentación Analítica, S.A. Madrid, Spain) on a homogenate of 10 g of sample in 100 mL distilled water.

2.4 Fatty acid profile.

Fatty acid composition of individual (olive, linseed and fish) oils and lipid mixture incorporated in oil-water emulsions were determined (in duplicate) by gas chromatography. Boron trifluoride/methanol was used for fatty acid methyl ester (FAME) preparation [22]. A Shimadzu gas chromatograph (Model GC-2014, Kyoto, Japan) fitted with a capillary column SP^{TM}-2330 (60 m x 0.25 mm x 0.2μm i.d.) (Supelco, Inc, Bellefonte, USA) was used with a flame ionisation detector (FID). Injector and detector temperatures were 250 and 260 ºC respectively, and the oven temperature was 140 ºC for 5 min, raised to 240 ºC at a rate of 4 ºC/min and held for 20 min. Fatty acids were identified by comparison with a known standard FAME mixture (Supelco, Alltech Associated, Inc., Deerfield, IL, USA).

2.5 Emulsion stability

Thermal emulsion stability [23] of the different samples was determined (in quadruplicate) as follows. The sample was stuffed into tubes, which were hermetically sealed

and heated in a waterbath for 30 min at 70 ºC; they were then opened and left to stand upside down (for 50 min) to release the separated fat and water onto a plate. Emulsion stability, as total fluid release, was expressed as % of initial sample weight. Creamy stability was determined (in quadruplicate) as visible phase separation in the plastic tubes containing the different samples after storage at 2 °C for 3 days. It was expressed as % of initial sample weight.

2.6 Instrumental colour measurement

The oil-in-water emulsions were removed from their plastic tubes and cut up. The colour of the cross-section was immediately measured by determining L*, a* and b* using a CIELab scale, where L* is the parameter that measures lightness, b* the tendency towards yellow and a* the tendency towards red. Measurements were performed on a Chroma Meter CR-400 (Konica Minolta Business Technologies, Inc., Tokyo, Japan). Determinations were performed in quintuplicate.

2.7 Penetration tests

Texture analysis was based on a penetration test [24] on the surface of oil-in-water emulsions (in plastictubes) at room temperature (22 8C). The test was performed using a 6 mm diameter cylindrical stainless steel plunger attached to a 5 Kg cell connected to the crosshead of TA-XT plus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY). The corresponding force-penetration curves (at 0.8 mm/s crosshead speed) were plotted and analysed. The rheological parameters of each sample derived from these curves were: distance at the point of gel rupture (D, mm); slope of the force-deformation curve (S, dimensionless), penetration force (PF, N), the force exerted at the point of gel rupture, and gel strength (GS, J), which is defined as the area enclosed by the force-deformation curve. Determinations were carried out six times.

2.8 Dynamic rheological properties

Dynamic rheological experiments were conducted using a controlled stress rheometer (Bohlin CVR 50, Bohlin Instruments, Gloucestershire, UK) at 25 °C with parallel plates (20 mm diameter and 1mm gap) and a solvent trap to prevent evaporation. Samples were allowed

to relax for 5 min before conducting rheological measurements such as equilibration time after loading the sample on the sensor system. Temperature control was carried out with a Peltier Plate system (-40 to +180 °C; Bohlin Instruments, Gloucestershire, UK). The linear viscoelastic region was determined for each sample through stress sweeps at 0.16 Hz. After that, a dynamic frequency sweep was conducted by applying a constant pre-determined stress within the linear region, over a frequency range between 0.01 and 10 Hz for each pre-emulsion. Viscoelastic properties (storage modulus, G´, loss modulus, G´´) were determined as functions of angular frequency (ω, rad/s) using the Bohlin software. Results reported were averages of three measurements.

2.9 Microscopy

The microstructure was analysed by scanning electron microscopy (SEM). Samples were fixed with a mixture (1:1 v/v) of paraformaldehyde (4 %) and glutaraldehyde (0.2 %) in 0.1 M phosphate buffer pH 7.2, post-fixed with OsO_4, washed, dehydrated in increasing concentrations of acetone, critical-point-dried, sputter-coated with gold/palladium in a metallizer (Balzer, SCD004) and scanned by SEM (Jeol, JSC,6400, Akishima, Tokyo, Japan) at 20 kV. Sample O/SC was not susceptible to SEM examination as its consistency was too weak for fixing. A large number of micrographs were taken and the most representative ones selected.

2.10 Statistical analysis

Each oil-in-water emulsion was prepared in duplicate. The effects of the replicates and of the different oil-in-water formulations was analyzed using the Statgraphics Plus 5.1 (STSC Inc. Rockville, MD, USA) package with a one-way ANOVA. When the effect of formulation was significant ($P < 0.05$), least-squares differences were used to compare the mean values and the Tukey-HSD test was used to identify statistically significant differences. Analysis of results between replicates showed that in all cases the respective two mean values for a given property were not significantly different ($P < 0.05$). Therefore tables of properties display the corresponding mean and SD values calculated from both duplicates in each formulation.

3 RESULTS AND DISCUSSION

3.1 Proximate analysis and fatty acid composition.

Proximate analyses generally differed little from one sample to another, and all were consistent with product formulation. The percentages of moisture and ash were around 42 % and 0.2-0.3 % respectively in all samples. Protein content ranged between 3.03 and 4.65 %; it was lowest ($P<0.05$) in O/SC (3.03 %) and O/SPI (3.36 %) samples. Protein content was 3.95 % in O/SC+MTG+MS and higher ($P<0.05$) in O/SC+MTG (4.17 %) and O/IPS+SC+MTG (4.65 %), but all presented comparable values ($P>0.05$). These differences were due to the stabilizing system used in the oil-in-water emulsions (Table 1) and hence to the presence of varying proportions of sodium caseinate, soy protein isolate or muscle protein. While this did not affect fatty acid composition, it did affect the nature of the protein matrix and hence the characteristics of the emulsion, as noted further below.

Fat content was around 53 % in all samples. The fatty acid profiles of individual oils (olive, linseed and fish) and healthier combination of them (used in oil-in-water emulsions) are reported in Table 2. Fatty acid composition differed for each type of oil. The fatty acid profile of the olive oil was characteristic of this kind of vegetable oil. It contained 15 % SFA, 75 % MUFA, with almost 72 % oleic acid, and 9 % PUFA. Olive oil is the most monounsaturated of all vegetable oils and has a high biological value, attributed to a high ratio of vitamin E to polyunsaturated fatty acids. It has a lower ratio of saturated to monounsaturated fatty acids than any other vegetable oil and contains antioxidant substances in optimum concentrations [14]. Olive oil intake is associated with a lessened risk of heart disease and breast cancer, and it has positive effects on colon cancer. Also, it has beneficial effects on postprandial lipid metabolism and thrombosis and inhibits LDL oxidation [25]. The linseed oil contained low levels of SFA (Table 2) and high levels of PUFA, 53 % of which were n-3 PUFA in the form of linolenic acid and 15 % n-6 PUFA in the form of linoleic acid. Fish oil is a very good source of n-3 PUFA (35 %), most of them long chain (33 %), with high levels of EPA (18.7 %), and DHA (12 %) acids (Table 2).

A unique combination of olive, linseed and fish oils was used to produce a lipid material with a fatty acid composition in line with health recommendations (Table 2). It contains a low proportion of SFA (less than 16 %), of which only 11 % (myristic and palmitic acids) have atherogenic properties [26]. Since these proportions are notably lower that those

found in meat fat (around 25 %) [26], the use of such oil-water emulsions in meat product reformulation can help to reduce the proportion of those fatty acids that increases the risk of cardiovascular disease (CVD).

The lipids in the oil-water emulsion contained high concentrations of MUFA (47.2 %), the principal MUFA being oleic acid (43.3 %) (Table 2). Typically, between 30 and 40 % of the fatty acids in meat fat are MUFA, and so the inclusion of such fat in meat product formulation will promote MUFA content. The presence of MUFA in the diet reduces the level of low density plasma lipoprotein-cholesterol [27]. The MUFA content of the oil-water emulsions was approximately 24 g/100 g, accounting for around 45 % of the total calories (approx. 490) in the emulsion. The PUFA content of the oil combination was 36 %, consisting of 26 % n-3 PUFA (5.29 % long chain and 20.7 % linolenic acid) and 10.2 % n-6 PUFA (Table 2). This makes the PUFA content close to 20 g PUFA (36 % of caloric value)/100 g of oil-in-water emulsion, of which 10 g was linolenic acid and 2.5 g/100 g EPA + DHA (4.5 % of caloric value). There is abundant evidence to suggest that regular consumption of and/or dietary supplementation with long chain n-3 PUFA (docosapentaenoic acid, EPA and DHA) confers a number of health benefits [3, 28]. Intake of these fatty acids is usually too low in western diets, and increased consumption of them is therefore recommended [3]. Daily ranges vary depending on several factors and run from 180 mg to 1000 mg for EPA and DHA [28] or 3-5.5 g for total n-3 PUFAs [29].

Since the health implications of fat consumption are determined by the proportions between fatty acids, some recommendations are still made on the basis of specific fatty acid ratios. Accordingly, the recommended ratio of PUFA/SFA should be > 0.4, and < 4 for the n-6/n-3 PUFA ratio [2, 26]. Excessive amounts of n-6 PUFA and very high n-6/n-3 PUFA ratios promote pathogenesis of many kinds, including CVD, cancer and inflammatory and autoimmune diseases, whereas increased levels of n-3 PUFAs (and low n-6/n-3 PUFA ratios) exert suppressive effects [3]. The oil combinations used in the oil-water emulsion formulation presented a PUFA/SFA ratio > 2, and a n-6/n-3 PUFA ratio of 0.4 (Table 2). PUFA/SFA and n-6/n-3 PUFA ratios in meat fat are naturally somewhat removed from the recommended values [26].

Hence, improved lipid content in meat based foods can be induced by incorporation of this kind of oil-water emulsion in product formulation. Depending on the level of animal fat

replacement, products can be made with smaller proportions of SFA and larger proportions of MUFA or PUFA (especially long chain n-3 PUFAs), better n-6/n-3 PUFA and PUFA/SFA ratios, and lower cholesterol contents.

3.2 pH, emulsion stability and colour

The pH value was highest ($P<0.05$) in the sample containing meat protein and MTG (O/SC+MTG+MS) and lowest in sample O/SPI (Table 3). All samples presented excellent fat and water binding properties, with no noticeable release of exudate during heating (thermal emulsion stability) and after 3 days of chilling storage (creamy stability). These are useful technological characteristics for purposes of meat product formulation. Although oil-in-water emulsions stabilized with SC and SPI have been widely used in the reformulation of many cooked and fermented meat products [7], there have been no studies on their stability. Colour parameters were generally little affected by oil-in-water formulation (Table 3). No changes ($P<0.05$) were observed in lightness values of the different samples, but the oil-in-water emulsions with the highest SC content showed lower redness values. Oil-in-water emulsion with SPI had the higher redness values (Table 3).

3.3 Penetration test

The penetration test results (Table 4) differentiate several types of behaviour in oil-in-water emulsions. In sample O/SC plunger penetration produced no breaking point and slope, PF and GS values were very low; in that case the system behaved like a viscous material but lacked a gel structure (gel-like behaviour). Sodium caseinate is commonly used as an emulsifier in the food industry because of its ability to rapidly confer a low interfacial tension and its strongly amphilic characteristics [30]. Sample O/SC is a type of oil-in-water emulsion commonly used to replace animal fat with a plant or marine fat in the formulation of cooked sausage [8, 13, 19] and fermented meat products [16].

In sample O/SPI there was again no breaking point, although PF and GS values were higher than in O/SC (Table 4); sample O/SPI would therefore appear to possess gel-like characteristics, if very weak ones. SPI are employed in a wide variety of food applications for their good technological properties, nutritional value and health benefits. Health claims by the FDA have resulted in increased consumer demand for products containing soy proteins [17].

SPI has been reported to form kinetically stable oil-in-water emulsions; it is known that these protein-covered oil droplets act as fillers, enhancing emulsion strength [31]. Such a system is stabilized by interactions occurring between the partially denatured proteins present in the dispersed phase [17]. Oil-water emulsions similar to sample O/SPI have been used, generally in the reformulation of fermented products [5, 16, 32].

The emulsions with MTG behaved quite differently (Table 4), with plunger penetration producing a breaking point similar to that of a gel structure. These emulsions presented breaking distances of less than 8.5 mm, the lowest values (maximum elasticity) occurring in sample O/IPS+SC+MTG. PF did not differ significantly among the samples containing MTG (O/SC+MTG, O/SC+MTG+MS, O/SPI+SC+MTG); the highest gel strength was observed in samples O/SC+MTG and O/SC+MTG+MS (Table 4). MTG activity and protein content help to explain differences in these emulsions properties. Gelling of an emulsion can be achieved by heating and enzymatic treatment. In cooked meat products it is based on a muscle protein thermal gelling process, which does not occur in uncooked (e.g. fermented) meat products. In both cases enzymatic cross-linking reactions offer new possibilities for improving the characteristics of oil-in-water emulsions for use as non-meat ingredients. MTG has been used to help stabilize emulsions made with SC and SPI [18]. In our experiment several emulsions were prepared with MTG (Table 1) to take advantage of the latter's ability to interact with some proteins. O/SC+MTG+MS, O/SC+MTG and O/SPI+SC+MTG samples presented clearly different penetration test parameters (Table 4) from those found in samples O/SC and O/SPI. Caseinate forms cross-links by means of MTG-forming polymers [33-35]; an interaction of this kind may have contributed to the fact that sample O/SC+MTG was more stiff and elastic than sample O/SC sample, without MTG (Table 4).

Various researchers have shown that MTG acts by cross-linking meat proteins at low temperature; it can therefore be used in meat processing to improve rheological properties while reducing, or even eliminating, the need for salts [36]. Caseinate has proven to be a good substrate for MTG [35], facilitating cross-linking between meat protein and caseinate molecules [33], which promotes the formation of a much more stable gel matrix at low temperatures. Meat proteins were therefore included in the emulsified-gelling systems (O/SC+MTG+MS) to modify the oil-in- water emulsion properties. However, when compared

with emulsion O/SC+MTG, the presence of meat protein produced no significant change in the penetration parameters (Table 4).

In the case of emulsion O/IPS+SC+MTG, the aim was to test the possibility of forming a combination SPI/SC gel/emulsion system, since both proteins are good substrates for MTG [18]. The combined use of caseinate and soy protein with MTG (O/SPI+SC+MTG) produced a gel/emulsion system with D and GS values lower ($P<0.05$) than other samples formulated with MTG, but with similar ($P>0.05$) PF values (Table 4).

3.4 Dynamic rheological properties

Figure 1 shows the storage and loss moduli (G´and G´´ respectively) of oil-in-water emulsions as functions of the angular frequency of oscillatory deformation. Note that the stabilization system of the oil-in-water emulsion affected its rheological behaviour. Except in O/SC, sample behaviour was predominantly more elastic than viscous, with G´ greater than G´´ throughout the ω interval studied; however, there are some differences between their patterns, which suggests differences in viscoelastic behaviour. O/SPI, O/SC+MTG, O/SC+MTG+MS and O/SPI+SC+MTG have similar profiles in which G´ presents a curve that is slightly dependent on frequency, while G´´ is practically independent of frequency; also, there is a tendency for minimum values to occur at intermediate angular frequencies in the case of samples O/SPI and O/SC+MTG, which has been attributed to a weak gel [37, 38]. O/SPI+SC+MTG was the sample that presented the strongest gel behaviour, with the highest values of G´ and G´´, which display practically no dependence on frequency (Fig. 1). It is well known that as the structural strength of such systems increases, the influence of the frequency diminishes, so that the gel structure is consolidated. However, the behaviour of sample O/SC was completely different (Fig. 1). At low frequencies G´´ was higher than G', indicating predominantly viscous behaviour up to intermediate frequencies where G´ and G´´ reversed places, with G´ subsequently presenting higher values than G´´. It is elastic behaviour that predominates in this range, although both moduli were strongly frequency-dependent. This means that samples behaved like a concentrate solution at low frequency and like a weak gel at higher frequencies.

The maximum rheological parameters measured (G´and G´´) were recorded in sample O/SPI+MTG+SC, with values as high as 7000 Pa (for G'), although the differences from the

other oil-in-water emulsions containing MTG (O-SC+MTG and O-SC+MTG+MS) were not great, the latter reaching 5600 and 5900 Pa respectively. The lowest G´ and G´´ values were recorded in the samples stabilized only with sodium caseinate (O/SC) or soy protein isolate (O/SPI). There are various factors that can help explain these differences in behaviour. The emulsions formed with only sodium caseinate or soy protein presented a concentrated solution-like and a weak gel-like structure respectively (Fig. 1) in which non-covalent weak reactions predominated. Since both emulsions had similar protein content, such differences may be attributable to variation in the specific activity of SC and SPI. The surface activities and emulsifying properties of soy proteins and casein have been extensively studied [30, 39, 40]. Even though soy proteins typically possess weaker emulsifying properties when compared to other surface-active proteins, soy proteins exhibit strong emulsifying properties compared to other plant proteins [41]. This difference is mainly due to the compact tertiary structure and the quaternary structure of the protein components present in soy protein concentrates and isolates [40].

With regard to the dynamic rheological behaviour of samples with MTG, there are two additional factors to be considered: protein content and MTG activity. Samples contained more protein, favouring protein-protein interactions. On the other hand, new covalent bonds are produced by transglutaminase-catalysed cross-linking [33-36], which would account for the formation of structures that were stronger, more elastic structures (with higher elastic and viscous moduli) and less dependent on frequency (Fig. 1). The result of this is an increase in protein-protein interactions, producing a stronger structure, as further borne out by the penetration test results (Table 4).

3.5 Microscopy

The morphological characteristics of oil-in-water emulsions were investigated using SEM. In these emulsions the oil phase was aggregated and entrapped in a structured network, although there were some differences in the organization of the network structure depending on the protein system used to stabilize the oil-in-water emulsions.

Sample O/SC presented a homogeneous structure (Fig. 2a). This oil-in-water emulsion contained a large number of cavities (left by fat, air and moisture) of various sizes (generally larger than those present in the other samples- Fig. 2b-e), round or oval in shape and evenly

distributed throughout the protein matrix. The high capillarity of this sample, associated with a denser but softer protein matrix, may help to explain why it behaved like a concentrate solution without exhibiting a gel-like structure as discussed previously.

The microstructure of oil-in-water emulsion stabilized with soy protein isolate (samples O/SPI and O/SPI+MTG+SC) contained clearly-observable soy protein structures (more evident in sample O/SPI) in the three-dimensional network formed (Fig. 2b and 2e). It has been reported that soy proteins aid in the formation of emulsions by reducing the interfacial tension between water and oil [42]. However, because of their globular aggregated structure, soy proteins do not unfold and adsorb at the interface, but rather form a thick interfacial layer which acts as physical barrier to coalescence [42, 43].

The MTG induced a cold-gelling process in oil-in-water emulsions, resulting in a more compact network structure with numerous small cavities (Fig 2c-e). As reported previously, protein content can influence these morphological characteristics as well as MTG activity. Both factors favour protein-protein interaction, which may have contributed to the elasticity and stiffness of MTG-induced gels noted above (Table 4 and Fig. 1) [18]. This microstructure seems to be associated with good binding properties in gels. No clear morphological differences were observed between samples O/SC+MTG (Fig. 2d) and O/SC+MTG+MS (Fig. 2c) as was the case in other gel characteristics (Table 3 and Fig. 1), indicating that the characteristics of oil-in-water emulsion stabilized with SC and MTG were hardly affected by the addition of meat protein. Elasticity (distance at the point of gel rupture) and gel strength (Table 4) were clearly affected by disorganization and loss of regularity in the matrix (Fig. 2 e) caused by the presence of soy protein.

4 Conclusion

Healthier lipid formulation based on processing strategies is one of the most important current approaches to the development of potential meat-based functional foods. Oil-in-water emulsion technology has been shown to be feasible as a means of stabilizing a mixture of vegetable (olive and linseed) and fish oils; these are combined in such a way as to produce an improved fatty acid profile with a low proportion of SFA, a high proportion of MUFA (oleic acid) and n-3 PUFA and a better balanced n-6/n-3 ratio. The physicochemical properties required of oil-in-water emulsions may differ according to the kind of meat product concerned.

By using any of various protein systems based on SC, isolated soy protein, microbial transglutaminase and meat protein as stabilizers, we canproduce healthier lipid emulsions with different properties, suitable for use as fat replacers in different types of new meat based-functional foods. The different physicochemical properties of such systems will determine their suitability for use as fat replacers in particular meat products. Work is currently in progress to assess the technological suitability of such healthier oil-in-water emulsions in different meat products.

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Conflict of interest statement

The authors have declared no conflict of interest.

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Table 1. Formulation [g] of different oil-in-water emulsions.

Samples	O†	Water	SPI†	MS†	MTG†	SC†
O/SC	789.47	631.58				78.95
O/SPI	789.47	631.58	78.95			
O/SC+MTG+MS	789.47	608.00		23.58	5.37	78.95
O/SC+MTG	789.47	631.58			5.37	78.95
O/SPI+SC+MTG	789.47	631.58	78.95		5.37	14.21

†O: Oil combination (44.39 % olive oil, 37.87 % linseed oil and 17.74 % fish oil); SC: sodium caseinate; SPI: soy protein isolate; MTG: microbial transglutaminase; MS: meat slurry.

Table 2. Fatty acid profile [as % of total fatty acids] and ratios of nutritional relevance of olive, linseed, and fish oils as well as the healthier oil combination used to prepare oil-in-water emulsions.

Fatty Acid	Olive Oil	Linseed oil	Fish oil	Oils combination
14:0	-	-	6.78±0.05	1.18±0.04
16:0	10.45±0.71	5.09±0.78	17.80±0.10	9.85±0.08
C18:0	3.99±1.04	4.66±0.02	3.55±0.01	3.91±0.02
C20:0	0.43±0.01	0.19±0.27	0.38±0.01	0.41±0.01
C21:0	-	-	2.79±0.01	0.47±0.01
∑ SFA	14.87±1.76	9.94±0.49	33.02±0.12	15.81±0.11
9c-16:1	0.68±0.00	-	7.68±0.07	1.64±0.04
9c-18:1	71.74±1.82	20.77±0.09	9.25±0.01	43.27±0.00
11c-18:1	2.90±0.04	0.97±0.00	3.07±0.02	2.24±0.06
∑ MUFA	75.32±1.78	21.74±0.09	20±0.04	47.15±0.03
18:2 n-6	8.65±0.26	15.23±0.06	1.25±0.04	10.15±0.02
18:3 n-3	0.32±0.02	53.08±0.33	1.06±0.02	20.77±0.15
20:5 n-3	-	-	18.76±0.03	3.04±0.02
22:5 n-3	-	-	2.07±0.01	0.32±0.01
22:6 n-3	-	-	11.98±0.06	1.93±0.05
∑ PUFA	8.97±0.24	68.31±0.39	35.12±0.10	36.21±0.17
∑ trans Fat Acids	-	-	3.29±0.03	0.38±0.00
PUFA/SFA	0.60	6.87	1.09	2.29
∑ n-3	0.32±0.02	53.08±0.33	33.86±0.06	26.06±0.07
∑ n-6	8.65±0.26	15.23±0.06	3.56±0.08	10.52±0.02
n-6/n-3	26.75±1.08	0.29±0.00	0.10±0.00	0.40±0.00

Results are expresed as mean ± standar desviation.

Table 3. Variation of pH value and colour parameters (lightness-L*, redness-a* and yellowness b*) of oil-in-water emulsions[†]

Samples	pH	L*	a*	b*
O/SC	7.94 ± 0.07b	89.36 ± 0.64a	-4.66 ± 0.15ab	21.85 ± 0.24a
O/SPI	7.80 ± 0.07a	87.87 ± 0.11a	-3.97 ± 0.06c	20.97 ± 0.09a
O/SC+MTG+MS	8.65 ± 0.09d	88.25 ± 0.28a	-4.36 ± 0.09b	21.51 ± 0.39a
O/SC+MTG	8.18 ± 0.04c	89.18 ± 0.67a	-4.83 ± 0.06a	22.12 ± 0.66b
O/SPI+SC+MTG	7.98 ± 0.07b	87.30 ± 0.39a	-3.90 ± 0.12c	20.81 ± 0.31a

[†] O/SC: oil-in-water emulsion prepared with sodium caseinate (SC); O/SPI: oil-in-water emulsion prepared with soy protein isolate (SPI); O/SC+MTG+MS: oil-in-water emulsion prepared with SC, microbial transglutaminase (MTG) and meat slurry (MS); O/SC+MTG: oil-in-water emulsion prepared with SC and MTG; O/SPI+SC+MTG: oil-in-water emulsion prepared with SPI, SC and MTG. Means ± standard desviation. Different letters in the same column indicate significant differences ($P<0.05$).

Table 4. Penetration test parameters of oil-in-water emulsions[†]

Samples	Distance [mm]	Slope [N/mm]	Penetration force [N]	Gel strength [mJ]
O/SC	10.00 ± 0.000c	0.001 ± 0.000a	0.01 ± 0.001a	0.06 ± 0,004a
O/SPI	10.00 ± 0.000c	0.012 ± 0.001a	0.18 ± 0.003b	1.41 ± 0,027b
O/SC+MTG+MS	7.84 ± 0.264b	0.173 ± 0.004c	1.40 ± 0.032c	6.16 ± 0,374d
O/SC+MTG	8.45 ± 0.354b	0.144 ± 0.008b	1.26 ± 0.033c	5.85 ± 0,357d
O/SPI+SC+MTG	5.61 ± 0.390a	0.226 ± 0.011d	1.29 ± 0.027c	3.37 ± 0,365c

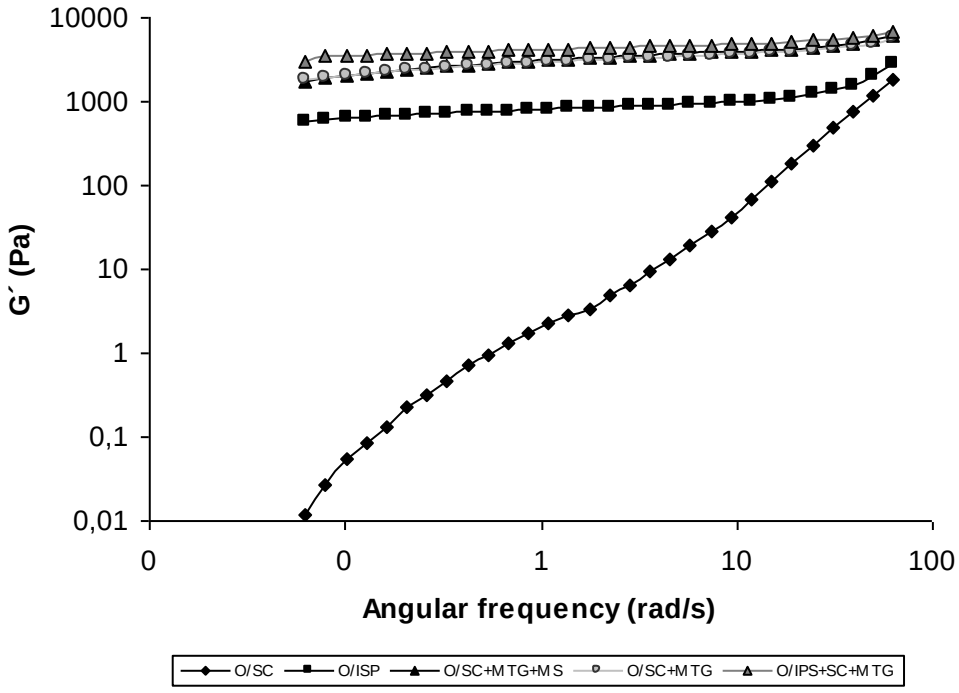
[†]O/SC: oil-in-water emulsion prepared with sodium caseinate (SC); O/SPI: oil-in-water emulsion prepared with soy protein isolate (SPI); O/SC+MTG+MS: oil-in-water emulsion prepared with SC, microbial transglutaminase (MTG) and meat slurry (MS); O/SC+MTG: oil-in-water emulsion prepared with SC and MTG; O/SPI+SC+MTG: oil-in-water emulsion prepared with SPI, SC and MTG. Means ± standard desviation. Different letters in the same column indicate significant differences ($P<0.05$).

Figure legends

Figure 1. Storage modulus (G') and loss modulus (G'') of oil-in-water emulsions. O/SC: oil-in-water emulsion prepared with sodium caseinate (SC); O/SPI: oil-in-water emulsion prepared with soy protein isolate (SPI); O/SC+MTG+MS: oil-in-water emulsion prepared with SC, microbial transglutaminase (MTG) and meat slurry (MS); O/SC+MTG: oil-in-water emulsion prepared with SC and MTG; O/SPI+SC+MTG: oil-in-water emulsion prepared with SPI, SC and MTG.

Figure 2. Scanning electron micrograph of oil-in-water emulsions: a) O/SC; b) O/SPI; c) O/SC+MTG+MS; d) O/SC+MTG; e) O/SPI+MTG+SC. O/SC: oil-in-water emulsion prepared with sodium caseinate (SC); O/SPI: oil-in-water emulsion prepared with soy protein isolate (SPI); O/SC+MTG+MS: oil-in-water emulsion prepared with SC, microbial transglutaminase (MTG) and meat slurry (MS); O/SC+MTG: oil-in-water emulsion prepared with SC and MTG; O/SPI+SC+MTG: oil-in-water emulsion prepared with SPI, SC and MTG.

Figure 1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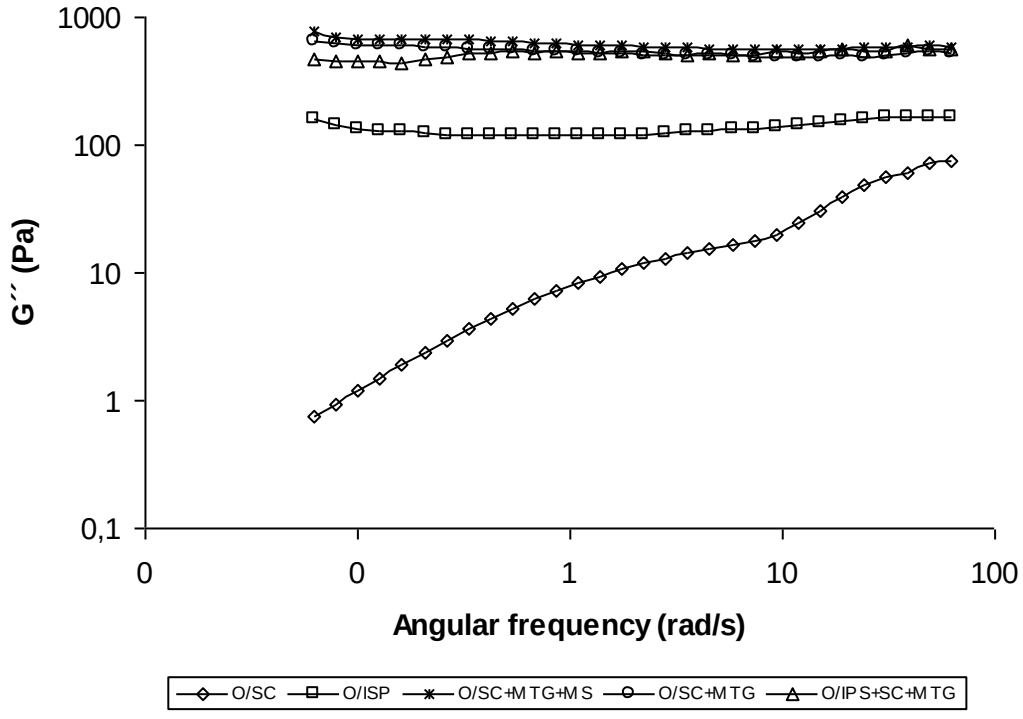
O/IPS+SC+MTG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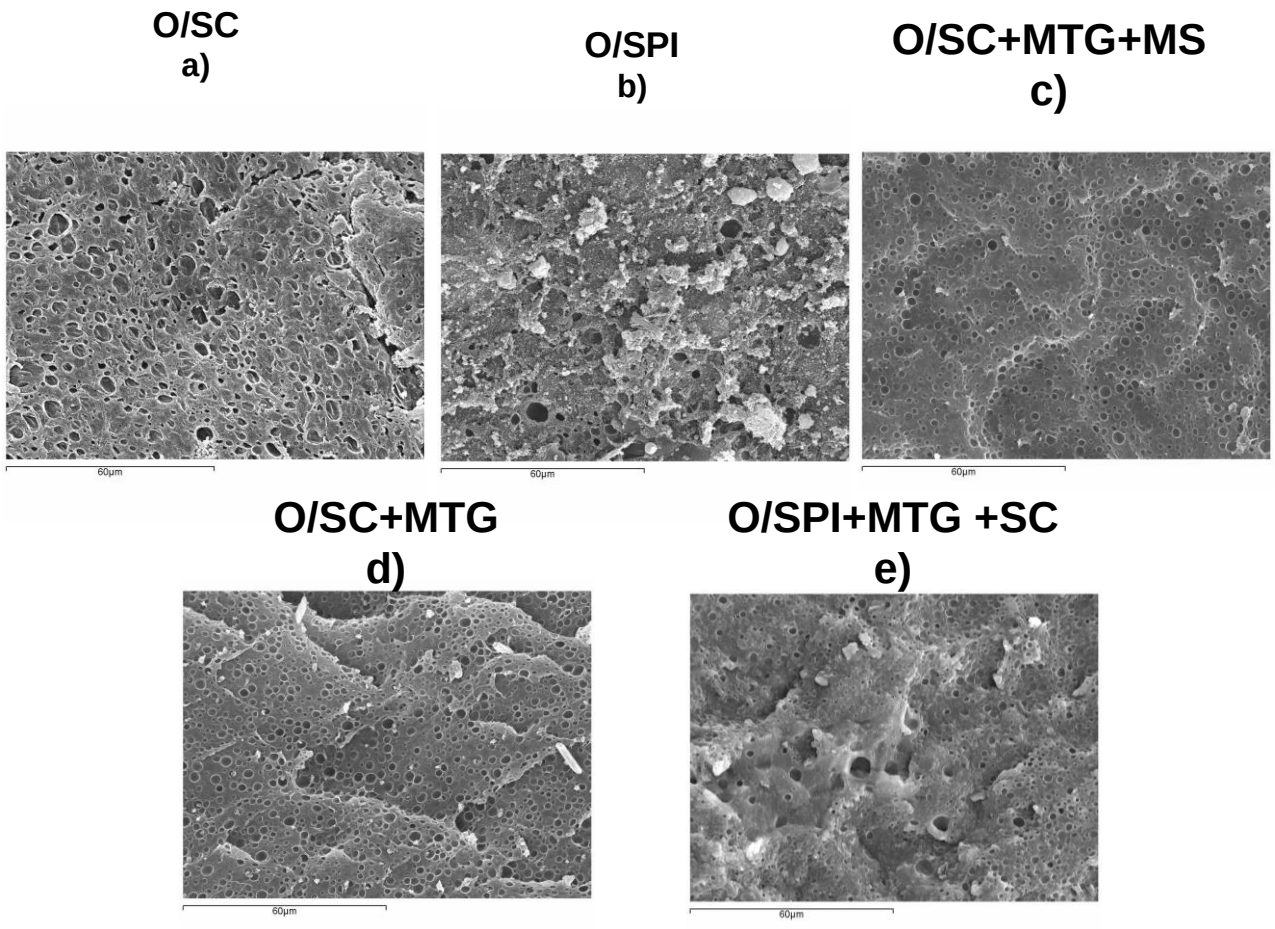
O/IPS+SC+MTG

Figure 2


O/SC
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c)
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e)
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