Omega-3 and Polyunsaturated Fatty Acids-Enriched Hamburgers Using Sterol-Based Oleogels

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Obesity and cardiovascular diseases are among the most worrying health problems worldwide. Dietary habits can be catalysts for the rise of these health issues in western countries. In this work, a meat product (pork patties) commonly elaborated with a high fraction of saturated fat is reformulated with an oleogel based on linseed oil (rich in polyunsaturated fatty acids). The oleogel is used for the partial replacement of the solid fat fraction present in pork patties (H-25 for 25% and H-75 for 75% of replacement). Incorporation of oleogels results in the modification of the fatty acid profile and in the significant decrease of the omega-6/omega-3 ratio. Results show that for both degrees of fat substitution, there are no differences between the patties produced with oleogel incorporation and the control, regarding textural parameters such as hardness, cohesiveness, and chewiness. Overall, samples with less amount of oleogel (H-25) are well classified in the acceptance and preference tests, despite the clear preference among the sensorial panel toward the control samples. These results show the feasibility of introducing oleogels as a fat replacer in the manufacturing process of pork patties, though there is still work to be done regarding some of their sensorial attributes.

Practical Applications: The purpose of this work is focused on the study of the properties of meat patties after the replacement of saturated fat with a multicomponent oleogel, foreseeing the hamburger production. The results show that the oleogel incorporation in meat patties is possible at the industrial level without additional unitary steps during meat patty production. Based on this work it is possible to produce meat patties with adjusted fatty acids profiles.

Foundation stated recently that at the moment of purchase, 64% of consumers consider health, when making a final decision, and the expected tendency is that this number increases in the next years.^[2] Moreover, an approximate number of 67% of consumers check for saturated fat in food products' labels and about 50% of all consumers are likely to purchase a product with a "no saturated fat" claim.^[3] So, consumers' perspectives on healthier food ingestion are directed toward the increase of the consumption of low-fat food products with higher contents of unsaturated fatty acids. Also, polyunsaturated fatty acids (PUFAs) like omega-3 and omega-6 are essential and can only be provided to the human organism through the diet (they cannot be synthesized by the human body due to lack of endogenous enzymes).^[4] In the past 30 years western countries' diets have changed resulting in an increasing consumption of omega-6 fatty acids and thus an unbalanced omega-6/omega-3 (n-6/n-3) ratio intake. This larger intake of omega-6 is directly associated to the prevalence of overweight and obesity.^[5] In addition, the intake of total fat (including saturated fat) as a percentage of the total calories ingested has continuously increased in the same period of time.^[5]

1. Introduction

Current consumption trends are directed toward natural healthy meat products.^[1] The International Food Information Council

Due to the appealing aspect (to most consumers) and versatility of meat products, there is a great interest in using them in a healthy diet with increased benefits to the consumers, as long as no major changes are inflicted in appearance and taste of the final

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product.^[6] In meat-related industrial processing there is a generalized use of numerous additives such as binders, fillers, emulsifiers and stabilizers. Their use is mostly linked to the induction of ingredient's stabilization, enhancement of flavors and also reduction of formulation costs.^[7] In that sense, solid-state lipids play an important role regarding the structure and taste of the meat products because these systems are responsible for conveying structural and meat binding properties that will influence the texture of foods. These solid lipids are traditionally composed by assembled networks of crystalline triacylglycerols (TAGs).^[8,9] It is well known that mouth-feel and textural properties like plasticity, cohesiveness, and hardness of many foods, including ice cream, chocolate, and processed meat products are conveyed by commonly used fats.^[10,11] In this case, the main problem is that saturated fats are directly associated with adverse effects on lipoprotein (cholesterol) profile as well as in cardiovascular diseases.^[12] Saturated fats are regularly introduced in a large scale in the processes as a natural-source substitute for *trans* fats. However, food industry companies have been suffering intense pressure to develop new healthier food formulations. Hamburger is a meat product regularly manufactured using lean meat, in conjugation with fat, that is frequently trimmed from subcutaneous origin.^[13]

One of the solutions to replace saturated fats in food products are oleogels (or organogels) that can be defined as a structure where an organic liquid is trapped inside of a thermoreversible 3D gel network.^[14,15] The feasibility of replacing fat content in meat products is somewhat dependent on the ability of oleogels to mimic the characteristics of the traditionally used fats. Also, replacing saturated fatty acids (SFAs) with PUFAs or monounsaturated (MUFAs) ones is certainly one of the main objectives of fat reduction in meats products.^[16–19] In this context, it is very important to understand how these oleogels will behave under different processing and storage conditions and how they will interact within the processed meats' structure.

In the current study, we used a multicomponent gelation strategy that comprises the use of linseed oil as the organic solvent and a mixture of plant sterols (γ -oryzanol and β -sitosterol) as oil gelators. Translating into a molecular scale, γ -oryzanol and β -sitosterol self-assemble by means of physical stacking of the two molecules on top of each other. The assembly of γ -oryzanol and β -sitosterol is characterized by a hollow tubular-shaped arrangement, eventually leading to gelation. The ferulic moiety that is present in the γ -oryzanol molecule will be responsible for a "crooked" stacking pattern, leading to the formation of hollow spiral-shaped fibrils (≈ 7.2 – 10 nm in diameter), which form a self-sustained and transparent organogel.^[20] This architectural arrangement will be responsible for the oil entrapment and consequently for the 3D final structure that is given to the oil phase. In terms of appearance and texture, sterol-based oleogels are quite transparent and reveal mechanical features similar to fat blocks.^[6,21] Besides providing structure to oil these gelators present health benefits when consumed.^[22] The European Food Safety Authority (EFSA) reported, under a health claim referring to disease risk reduction, that the minimum duration that is required for achieving the maximum effect of plant sterols and stanols on LDL cholesterol lowering is 2–3 weeks.^[23] These oleogels will also modify the fatty acid

profile of the hamburgers due to the use of linseed oil, which is rich in n-3 (alpha linolenic acid) and n-6 (linoleic acid) fatty acids.^[24]

In this work, sterol-based oleogels were added to pork patty for the replacement of fat. Two levels of saturated fat replacement were tested and the produced pork patties were evaluated in terms of physical and chemical characteristics and by comprehensive consumer and acceptance sensorial tests.

2. Experimental Section

2.1. Raw Materials and Oleogel Production

An approximated amount of 4.50 kg of lean pork meat was used for all the experiments. Both lean pork meat and pork back-fat were purchased from a local butcher (Ourense, Spain). A commercial hamburger mixture that comprised salt, maize, dextrose, spices, sodium sulfite, ascorbic acid, sodium citrate, and cochineal colorant, was purchased from Ceylan (Manufacturas Ceylan S.L., Valencia, Spain). For the production of sterol-based oleogel, a commercial linseed oil Vitaquell (Hamburg, Germany) with 72% PUFAs (approx. 55% of α -linolenic), 19% MUFAs and 9% SFAs was used as the oil phase. A mixture with 60:40 (w/w) oryzanol-sitosterol ratio was used for the production of sterol-based oleogels. This ratio corresponds to $1:1 \text{ mol L}^{-1}$ of each component and was reported as the ratio that allows obtaining the firmest transparent gel.^[25] Oryzanol and β -sitosterol were purchased from Oryza Co. (Japan) and Sigma-Aldrich (France), respectively. Oleogels with 8% (w/w) of structuring agents were produced and used for all the experiments. Both structuring agents (γ -oryzanol and β -sitosterol) were dispersed under stirring until solubilization in linseed oil at 80°C , for 30 min as presented elsewhere.^[26] After that, the mixture was left cooling at room temperature until gel formation occurred.

2.2. Pork Patties Elaboration

For the hamburgers production, the lean pork meat and the subcutaneous pork fat were ground through a 6 mm diameter mincing plate in a refrigerated mincer machine (La Minerva, Bologna, Italy) and mixed in a vacuum mincer machine (Fuerpla, Valencia, Spain) for 3 min. The commercial hamburger mixture was added during mixing in order to homogenize all the components. Three sets of samples of patties were elaborated: a control set of samples (H-Co) and two sets were produced partially replacing subcutaneous pork fat with sterol-based oleogels in the following proportions: 25% (H-25) and 75% (H-75). For that, the correct amount of oleogel was added to the mixture and properly mixed until a paste was obtained. With the support of a burger-maker (Gaser, A-2000, Girona, Spain), the patties were shaped from the previously obtained paste. For every set of samples, 10 replicates were used for each of the performed measurements. **Table 1** presents the formulations of each set of samples and **Figure 1** shows the processing of the hamburgers.

Table 1. Formulation of pork patties: H-Co, control sample; H-25, with 25% of pork fat replacement; H-75, 75% of pork fat replacement.

Ingredients	H-Co	H-25	H-75
Lean pork meat [g]	1410	1410	1410
Subcutaneous pork fat [g]	90	67.5	22.5
Sterol-based oleogel [g]	—	22.5	67.5
Commercial burger additive [g]	42.3	42.3	42.3
Water [mL]	155	155	155

2.3. Physicochemical Composition

The pH of the burgers was measured using a digital portable pH-meter (HI 99163, Hanna Instruments, Eibar, Spain) equipped with a penetration probe. Color parameters were measured using a portable colorimeter (Konica Minolta CM-600d, Osaka, Japan) with pulsed xenon arc lamp, 0° viewing angle geometry and 8 mm aperture size, to estimate patties color in the CIELAB space: lightness, (L^*); redness, (a^*); yellowness, (b^*). The color was measured in three different points of each sample in homogeneous and representative areas.

Moisture, protein, and ash were quantified according to the ISO-recommended standards (ISO 1442, 1997; ISO 937, 1978; and ISO 936, 1998, respectively). Total fat was extracted according to the AOCS Official Procedure Am 5-04.^[27]

2.3.1. Fatty Acid Composition

The fat was extracted from 10 g of patty using 18 mL of chloroform and 20 mL of methanol, using an IKA T25 digital ultraturax. 10 mL of NaCl 1% (w/w) was added to enhance phase separation, with the sample being subsequently centrifuged at $4000 \times g$ for 10 min. Chloroform was recovered and fat was extracted through evaporation under vacuum at 56 °C in a rotary evaporator. Extracted fat was stored at −80 °C until fatty acid methyl esters (FAMES) analysis. According to the procedure

of Dominguez et al.,^[28] 50 mg of fat was used to determine the fatty acid profile. Separation and quantification of FAMES were done using a gas chromatograph (GC-Agilent 6890 N; Agilent Technologies Spain, S.L., Madrid, Spain) equipped with a flame ionization detector following the chromatographic conditions described, by Dominguez et al.^[28] FAMES composition was expressed in percentage of area with respect to identified total fatty acids.

2.3.2. Texture Analysis

Pork patties of 100 g were placed inside vacuum plastic bags and cooked in a water bath with automatic temperature control (JP Selecta Model Tectron Bio, Spain) at 70 °C. This target temperature was controlled by thermocouples type K (Comark, PK23M, UK) connected to a data logger (Comark Diligence EVG, N3014, UK). After sous vide cooking, pork patties were cooled at room temperature by placing the bags under water at 18 °C for at least 30 min.

Texture profile analysis (TPA) was performed in seven patty slices of $1 \times 1 \times 1$ cm (height $\times$ width $\times$ length) with a texture analyzer (TA.XTplus, Stable Micro Systems, Vienna Court, UK). The test was developed according to the methodology proposed by Bourne, Kenny, and Barnard (1978). Textural parameters were measured by compressing to 80% using a compression probe of 19.85 cm² of surface contact. The speed to record the force-time curves was 1 mm s^{−1}. Between the first and second compressions, the probe waited for 2 s. The computer software Texture Exponent 32 (version 1.0.0.68, Stable Micro Systems, Vienna Court, UK) was used to obtain hardness (N), cohesiveness, springiness (mm), and chewiness (N mm).

2.4. Consumer Sensorial Test: Acceptance and Preference

This study was performed with the objective of evaluating consumers' acceptance and preference of distinct food products. All samples used for sensorial evaluation were received at the

Figure 1. Representation of the hamburger production process.

Table 2. pH and chemical composition of pork patties elaborated with linseed oleogel.

	H-Co	H-25	H-75
pH	6.44 ± 0.04 ^a	6.62 ± 0.12 ^a	6.45 ± 0.13 ^a
Protein [%]	18.01 ± 0.22 ^a	17.74 ± 0.34 ^a	17.30 ± 0.24 ^a
Moisture [%]	73.09 ± 0.64 ^{a,b}	72.49 ± 1.01 ^{a,b}	72.30 ± 1.01 ^{b,c}
Fat [%]	2.83 ± 0.69 ^a	3.29 ± 0.45 ^{a,b}	4.05 ± 0.94 ^b
Ash [%]	2.61 ± 0.47	2.76 ± 0.96	2.45 ± 0.05

Different letters (in the same row) mean that samples are statistically different ($\alpha = 0.05$).

sensory analysis laboratory with proper identification. All tests were carried out following the UNE-EN ISO 8589:2010 normative. All patties were cooked using a convective oven, until the samples reached 70 °C. The sensorial evaluation was performed with 16 panelists from the Meat Technology Centre (seven female, nine male). After sampling, panelists used crackers without salt and water to clean the palate.

For the acceptance test, the sensorial evaluation was based in the method UNE-ISO 8587:2010. The attributes were evaluated using a hedonic 7-point scale (very much liked; liked a lot; liked; neither liked nor disliked; disliked; much disliked; very much disliked) in this manner the panelists described their felling regarding each hamburger sample. In the preference test, the panel classified each sample according to sensory attributes (general appearance; smell; tenderness; juiciness; taste; global evaluation). The visual appearance was classified taking into account the uncooked patty samples. The evaluations were made in different order of samples to the Friedman statistical test, which is a non-parametric test for evaluating the difference between several related samples. This test is used when the same parameter has been measured under different conditions on the same subjects, the grade of 1 was given to the preferred sample, grades 2 and 3 were given, respectively, for the second and the third most preferred samples.

2.5. Statistical Analyses

The statistical analyses were performed by means of analysis of variance using Tukey's mean comparison test (* for $p \leq 0.05$; ** for $p \leq 0.01$; *** for $p \leq 0.001$; **** for $p \leq 0.0001$) from average results and standard deviation (GraphPad Software, Inc. USA). For the sensorial results the Friedman statistical test was used to understand if there were statistical differences among the classifications given by the panelists ($\alpha = 0.05$).

3. Results and Discussion

3.1. Physicochemical Properties

3.1.1. Chemical Composition

The chemical composition of all sets of pork patties are presented in Table 2. Figure 2 shows the results of the total cholesterol content and the complete fatty acid profile composition of control

samples (H-Co) and samples with oleogel incorporation (H-25 and H-75).

It is possible to verify that total cholesterol (TC) content increased for a higher fat substitution. The similar molecular structure of plant sterols that are added through oleogel mass (that comprises plant sterols as gelator molecules) explains the observed TC values. With the higher substitution of pork fat and the incorporation of oleogel, this concentration increased and consequently higher values of TC were obtained. On the other hand, the content of SFAs and MUFAs decreased significantly for higher amounts of oleogel used; this oleogel contains linseed oil, thus contributing to the increase of PUFAs content. Changes of the fatty acid profile showed the decrease of the saturated fraction in samples with oleogel incorporation (H-25 and H-75) such as C18:1 oleic acid, and an increase of n-3 PUFA family such as α -linolenic acid (ALA; 18:3n-3). These changes are explained by the fatty acid profile of linseed oil that is the main component of the oleogels used to replace pork fat.

It was demonstrated that, as a consequence of different treatments, statistically different n6/n3 ratios were obtained, leading to an increase of the healthier cholesterol fraction in the final product (after replacing pork fat with sterol-based oleogels). A decrease of the ratio of LDL/HDL in a diet with hamburgers with higher incorporation of oleogel is also expected. The positive health effects of omega-3 have been credited to ALA elongation and denaturation products like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). A great importance is given to dietary fatty acids and their role in pathogenesis, but also in the preventive effect of metabolic syndrome.^[29]

3.1.2. Texture Analysis and Color

Texture analysis, performed by a compression test, evaluated the effect of oleogel incorporation in the hamburgers samples that were obtained by sous vide cooking procedure of the meat patties. Results showed that according to oleogel incorporation (pork fat substitution degree) the hamburger samples H-75 present a statistically different behavior regarding the chewiness parameter when compared with the control samples (H-Co). The increase of oleogel in the samples from 25% to 75% of the total fat also influenced the hardness of the samples. The tendency for hardness to decrease with the increase of fat replacement percentage is in accordance with the values reported by Moghtadaei et al.^[30] Despite that, our results showed similar values between the control samples and the samples H-25. This is indicative of the fact that the reformulation of the internal meat product structure did not produce a significant weakening structure effect. As demonstrated by Zetzel et al.^[31] the incorporation of an ethylcellulose-based oleogel as fat replacer in frankfurters allowed to maintain the microstructural arrangement of the fat droplets preventing a negative effect on overall textural results that could occur if fat droplet sizes would get too small. Among the other samples, no statistical differences were observed (see Figure 3). These results are in line with the values obtained for the tenderness parameter that was evaluated during the comprehensive preference test. The oleogel itself presented an average hardness value of 9.921 ± 0.723 (N) and an adhesiveness value of -0.428 ± 0.089 (N s). No differences were observed

Figure 2. Fatty acid profile of patty samples: H-Co, control sample; H-25, with 25% of pork fat replacement; H-75, 75% of pork fat replacement (TC, total cholesterol; SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids; PUFA, polyunsaturated fatty acids; n6/n3, ratio of omega-6/omega-3).

for the adhesiveness when oleogel is added to the hamburger. Oleogel incorporation and consequent subcutaneous fat removal did not result in a partial disintegration of the structural configuration of the hamburger samples. The results led us to conclude that this oleogel is indeed a very good replacer for regular fat, once the textural parameters obtained could guarantee similar organoleptic properties and the stability of the hamburgers. Despite that, mixtures of γ -oryzanol and β -sitosterol are known to be susceptible to crystalline rearrangement when water activity is increased.^[32] This must be taken into account when considering the final products long-time exposures to such environmental conditions during storage, once the oleogel's structure could risk destabilization.

Statistical differences ($p < 0.05$) were observed in the color of cooked hamburgers with the addition of the oleogel (Table 3). The color represents a critical objective quality parameter for quality index measurements of raw and processed foods. The cooking process of meat denatures myoglobin, leading to the characteristic (dull) brownish color of cooked meat. The uniformity within the complete set of samples showed a significant increase in the

lightness value, reducing the browning effect. It has been reported that meat batters, produced with vegetable oils as an ingredient for fat substitution, revealed decreasing lightness and redness values.^[33] This is not in agreement with our results, which is explained by the glassy appearance of the oleogels that is responsible for a gradual lightness increase as the amount of oleogel increases.

3.2. Sensorial Tests

3.2.1. Acceptance Test

The results of the sensorial evaluation (presented in Figure 4) performed after tasting control hamburger samples (H-Co), showed that, from the 16 members of the sensorial panel that participated in the acceptance test, three of them selected "very much liked," 10 of them selected "liked a lot" and three pointed to "liked." Overall, the fat substitution with sterol-based oleogels was not received negatively by the consumers. For the sample with the

Figure 3. Textural analysis of hamburger samples: H-Co, control sample; H-25, with 25% of pork fat replacement; H-75, 75% of pork fat replacement.

Table 3. Color parameters (L^* , a^* , b^*) and RGB conversion of hamburger samples; H-Co, control sample; H-25, with 25% of pork fat replacement; H-75, 75% of pork fat replacement. RGB conversion was performed using Matlab software.

CIE-lab	H-Co	H-25	H-75
L^*	45.513 ± 2.744^a	49.773 ± 2.889^b	53.409 ± 2.050^c
a^*	16.990 ± 1.045^a	18.896 ± 1.828^a	18.248 ± 0.950^a
b^*	14.624 ± 0.901^a	$17.042 \pm 0.797^{b,c}$	$16.816 \pm 1.412^{c,b}$
RGB conversion	H-Co	H-25	H-75
R	142	158	167
G	97	106	115
B	84	90	100
Color

Different letters (in the same row) mean that samples are statistically different ($\alpha = 0.05$).

least amount of fat replacement (H-25), two members of the jury selected “very much liked,” three selected “liked a lot,” four panelists chose “liked,” three of them preferred to mention “neither liked nor disliked” and four selected “disliked.” The sample with the higher oleogel incorporation (H-75) received one selection as “very much liked,” six selections as “liked,” four selected “neither liked nor disliked,” four selected “disliked” and finally one single selection as “much disliked.” The general acceptance test allowed us to classify the samples H-Co, H-25, and H-75, respectively, with the scores 2.00, 3.25, and 3.81.

Figure 4. Global acceptance results of hamburger samples: H-Co, control sample; H-25, with 25% of pork fat replacement; H-75, 75% of pork fat replacement.

3.2.2. Preference Test

The consumer preference test consisted in the evaluation of the hamburgers regarding six parameters: visual aspect of uncooked hamburgers, smell, tenderness, juiciness, taste and global evaluation. Regarding the visual aspect of the uncooked (raw) samples, from the sixteen participants nine (56%) selected the control sample (H-Co) as the preferred sample, five (31%) of them selected the sample H-25 and only two of them (13%) selected the sample H-75. All the preference test results are presented in **Figure 5** and it is possible to observe that the control sample (H-Co) was the number one preference for all the parameters and only the juiciness presented a similar preference to the sample H-25.

The results of the survey resulted in a nominal evaluation for every parameter tested. The consumers evaluated the preferred sample with a score of 1, the second preferred with 2 and least favorite with a 3 rating. This classification allowed to perform a Friedman Test, in order to understand how differently was the perception of the consumers of these three sets of hamburger samples. However, statistical analysis showed that for some of the parameters, no significant differences were observed. From the data obtained through Friedman analysis, provided in **Table 4**, we can identify that globally the samples were rated as different (global valuation). However, the control sample had no statistical differences ($p > 0.05$) for raw visual aspect, tenderness, juiciness and flavor when compared to H-25 samples. This means that trained panelist did not differ hamburger samples with lower amount of oleogel (H-25) from the control samples.

4. Conclusions

The introduction of oleogels in the pork patties showed to be a very promising approach, with interesting outcomes toward the fat substitution in meat-based products. Fat is indeed the main contributor in terms of physical and sensory attributes. Two levels of pork fat substitution were used and the results showed that it is possible to improve the fatty acid profile of these food products without changing most of the textural parameters evaluated. Regarding the consumer acceptance and preference there was a clear preference toward the control hamburger samples. Nevertheless, the hamburgers samples with less amount of oleogel (H-25) were placed in a very favorable classification among the sensorial panel in both the acceptance and preference tests. The

Figure 5. Results from the preference test for all the evaluated parameters.

Table 4. Friedman test results using valuation of 1, 2, or 3 for the preferred sample, second preferred sample, and for the third, respectively.

	H-CO	H-25	H-75
Raw visual aspect	25 ^a	30 ^{a,b}	41 ^b
Smell	20 ^a	40 ^b	36 ^{a,b}
Tenderness	26 ^a	30 ^{a,b}	40 ^b
Juiciness	29 ^a	29 ^a	38 ^a
Flavor	20 ^a	36 ^{a,b}	40 ^b
Global valuation	19 ^a	37 ^b	40 ^b

$F(\text{test}) > F(\alpha = 0.05)$. Different letters mean that samples (same line) are statistically different ($\alpha = 0.05$).

introduction of sterol-based oleogels as replacing agents for common fat in hamburgers is a strong possibility because the panelists hardly distinguished overall quality in H25, so it is expectable that consumers will not distinguish at all. These results demonstrated the feasibility of introducing oleogels as successful fat replacers in the manufacturing process of hamburgers. While the many benefits of the oleogel product are encouraging, whether they justify the costs involved in production is also important. The costs that are associated with producing the meat patties with oleogel are mainly determined by three of the ingredients that are used in the process (lean meat, type of oleogel and animal fat). Stipulating the price per gram for lean meat, oleogel and fat being, respectively, \$0.011, \$0.032, and \$0.0039, using 453 g as reference for the weight of the lean meat in one patty (that also contains 30% of fat content, being the water and additives 8% and 2.15% of the total patty mass) the cost per pound of an approximate 25% of substitution with oleogel is equal to the current market price. It would be encouraging to point out to potential customers by showing that incorporating oleogels has little to no extra costs for production and implementation. In the future, it is important to understand which are the levels of n-3 fatty acids that can withstand higher temperatures as a result of different cooking methods and how this would influence n-3 bioaccessibility through food consumption.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

oleogels, meat products, saturated fats, fatty acids, phytosterols

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