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Designing a Clean Label Sponge Cake with Reduced Fat Content.

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UNIVERSIDAD POLITECNICA DE VALENCIA

DEPARTAMENTO DE TECNOLOGÍA DE ALIMENTOS

Dr. Youling Xiong
Journal of Food Science

Dear Editor,

Please find enclosed a copy of the manuscript entitled "**Designing a clean label sponge cake with reduced fat content**" which we would like to be considered for publication in the **Journal of Food Science**.

In this study a natural functional ingredient derived from flaxseed, OptiSol™5300, is used as fat replacer. This ingredient is high in fiber and has hydrocolloid functionality. In this research, functional cakes with reduced fat content and good acceptability by consumers are obtained avoiding E-numbers on their labels.

We hope that the paper will be suitable for publication.

Thank you in advance,

Yours sincerely

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Designing a Clean Label Sponge Cake with Reduced Fat Content.

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5,060 words

Low fat cake with functional ingredient…

Food Chemistry

ABSTRACT

The fat in a sponge cake formulation was partially replaced (0, 30, 50, 70%) with OptiSol™5300.This natural functional ingredient derived from flax seeds, rich in fiber and alpha-linoleic acid, provides a natural substitute for guar and xanthan gums, avoiding E-numbers on labels. The structure and some physicochemical properties of the formulations were examined, sensory analysis was conducted and changes in starch digestibility due to adding this ingredient were determined. Increasing quantities of OptiSol™5300 gave harder cakes, with less weight loss during baking, without affecting the final cake height. There were no significant differences ($p > 0.05$) in texture, flavor and overall acceptance between the control and the 30% substitution cake, nor in the rapidly digestible starch values. Consequently, replacing up to 30% of the fat with OptiSol™5300 gives a new product with health benefits and a clean label that resembles the full-fat sponge cake.

Keywords: cake; flaxseed; fat replacer; structure; clean-label.

Practical Application: Sponge cakes with a functional flaxseed ingredient as fat replacer were obtained in this work. This ingredient, like oil, is distributed uniformly through the batter matrix. Cakes with 30% fat replacement were very well accepted by consumers and could be prepared and sold by food industry as clean-label reduced-fat cakes.

1. INTRODUCTION

Bakery products are very high consumption foods throughout the world. Sponge cakes are particularly popular and consumers consider them delicious products with certain particular organoleptic characteristics (Matsakidou and others 2010). Cakes contain approximately 15-25% fat (Matsakidou and others 2010). Fat influences the sensory and texture characteristics of the food product and, consequently, its acceptability to consumers. In sponge cakes, fat not only provides flavor but also makes it easier to incorporate air into the batter, contributing to its increase in volume, and interferes with the continuity of the gluten structure, favoring the formation of a softer cake (Psimouli and Oreopoulou 2013).

In developed countries, food-related diseases are increasing because, among other factors, energy ingestion is too high and the consumption of dietary fiber is below the recommended levels (Zahn and others 2010). Fat is the food constituent with the highest energy value (Zahn and others 2010). Excessive fat consumption is associated with obesity, cardiovascular problems and different types of cancer (Román and others 2015). A promising way for the food industry to supply healthier foods could therefore be to replace fat with dietary fiber in food formulations (Zahn and others 2010). Various studies have been published on reducing the fat content of cakes and muffins by adding different carbohydrates and fibers, such as inulin (Zahn and others 2010; Rodríguez-García and others 2012; Psimouli and Oreopoulou 2013; Rodríguez-García and others 2014b), β-glucan (Lee and others 2005; Kalinga and Mishra 2009), oat bran and flaxseed flour (Lee and others 2004), cocoa fiber (Martínez-Cervera and others 2011), polydextrose (Kocer and others 2007), maltodextrin (Lakshminarayan and others 2006; Psimouli and Oreopoulou 2013) and citrus pectin (Psimouli and Oreopoulou 2013). Few references to fat replacement by gums appear to have been published. Zambrano and others (2004) used guar and xanthan gums to obtain low-fat sponge cakes, Khouryieh and others (2005) prepared muffins in which they replaced fat with a mixture of maltodextrin and xanthan gum and Kaur and others (2000) used guar gum and carboxymethyl cellulose to reduce the fat content of muffins.

The importance of gums lies in their ability to control the rheological characteristics of an aqueous system. They can also influence the stabilization of emulsions and help to suspend particles, control crystallization and inhibit syneresis (Zambrano and others 2004).

In the present study, a functional ingredient with hydrocolloid capacity derived from flax seeds was used as a fat substitute. This ingredient can be used to replace guar and xanthan gums and, being a natural product, allows clean labeling of the food, avoiding E-numbers.

Flax seeds are a potential source for functional food formulations (Marpalle and others 2014). They are rich in health beneficial nutrients (Shearer and Davies 2005) and present a unique nutritional profile with a high concentration of polyunsaturated fatty acids (73% of the total fatty acids), a moderate concentration of monounsaturated fatty acids (18%) and a low concentration of saturated fatty acids (9%). Linoleic acid comprises 16% and alpha-linolenic acid (ALA) 57% of their total fatty acids (Marpalle and others 2014). ALA is an omega-3 fatty acid which is involved in reducing inflammatory processes and plays an important role in reducing/preventing cardiovascular diseases, osteoporosis, diabetes and digestive system diseases (Mercier and others 2014). Flax seeds contain approximately 28% fiber, of which a third is soluble. Soluble fiber is associated with the ability to reduce cholesterol and regulate blood sugar levels. The rest of the dietary fiber is insoluble. Insoluble fiber favors increased stool bulk and reduces bowel transit time, so it helps to prevent constipation and may provide protection against colon cancer (Moraes and others 2010).

The aim of this study was to assess the effect on the structure and the physicochemical and sensory properties of sponge cakes of using a natural functional ingredient derived from flaxseed to replace part of the fat in their formulation. A further aim was to study changes in starch digestibility as a result of adding this ingredient to the sponge cake formulation.

2. MATERIALS AND METHODS

2.1 Ingredients

The ingredients used in the preparation of the cake batters were: wheat flour (Harinas Segura S.L, Torrente, Spain, composition provided by the supplier: 13.50-15.50 g/100 g moisture, 9-11 g/100 g proteins); sugar (AB Azucarera Iberica S.L.U., Madrid, Spain); liquid pasteurized egg white and yolk (Ovocity, Llombay, Spain); skim milk (Corporación Alimentaria Peñasanta, S.A., Siero, Spain); refined sunflower oil (Aceites del Sur-Coosur, S.A., Vilches, Spain); OptiSol™5300 (natural ingredient derived from flaxseed; Glambia Nutritionals Ltd, Kilkenny, Ireland; specifications provided by the supplier: 28-36 g/100 g protein, 8-12 g/100 g moisture, 15-20 g/100 g fat, 30-40 g/100 g carbohydrate, 25-37 g/100 g dietary fiber) sodium bicarbonate and citric acid (Sodas y Gaseosas A. Martínez, S.L., Cheste, Spain); salt and distilled water. Oil was used instead of shortening as usually done in Mediterranean countries (Rodriguez-Garcia and others, 2012; Martinez-Cervera and others 2011)

2.2 Batter and cake preparation

Four formulations were prepared: the control formulation (R0) and three formulations (R30, R50 and R70) where fat was replaced at 30%, 50% and 70% respectively, by increasing amounts of OptiSol™5300 (Table 1). The fat content of OptiSol™5300 was taken into account to calculate the level of fat replacement in the different formulations. In order to achieve the proper dispersion for OptiSol™5300 to act as a fat mimetic, the appropriate amounts of water were added for an OptiSol™5300-to-water ratio of 1:5.

The batters were prepared according to the all-in mixing procedure proposed by Rodríguez-García and others (2014a), with some modifications. The liquid eggs, milk and water were placed in a Kenwood Major Classic mixer (Havant, England, UK). The dry ingredients (wheat flour, sugar, OptiSol™5300, sodium bicarbonate and citric acid and salt) were added to the liquids, and the oil was finally placed on the top. The mixing proceeded using a wire whisk at speed 1 for 30 sec, followed by 1 min at speed 2 and 3 min at speed 3.

For cake preparation, a conventional oven (Electrolux, EOC3430DOX, Stockholm, Sweden) was preheated to180 ºC for 30 min, and the batters (700g) were placed in a Pyrex baking pan (diameter = 20 cm) and baked at 180 ºC for 43 min. The cakes were then kept at room temperature for at least 1 h 30 min before being analyzed. All the batters and cakes were prepared in triplicate and the analyses were performed within 24 hours of their preparation.

2.3 Confocal laser scanning microscopy (CLSM)

Equipment and dyes. A Nikon confocal microscope C1 unit fitted on a Nikon Eclipse E800 microscope (Nikon, Tokyo, Japan) was used. An Ar laser line (488nm) was employed as the light source to excite the fluorescent dyes: Rhodamine B and Nile Red. Rhodamine B (Fluka, Sigma-Aldrich, St. Louis, Mo., U.S.A), with $\lambda_{ex\,max}$488 nm and $\lambda_{em\,max}$ 580 nm, was solubilized in distilled water at 0.2 g/100 mL. This dye was used to stain proteins and carbohydrates. Nile Red (Fluka, Sigma-Aldrich), with $\lambda_{ex\,max}$ 488 nm and $\lambda_{em\,max}$ 515 nm, was solubilized in PEG 200 at 0.1 g/L and used to stain fat. The objective lenses were 40x/1.0 and 60x/1.40 NA/Oil/ Plan Apo VC Nikon.

Sample viewing. A microscopy slide was prepared with 2 razor blades stuck to the glass (Alava and others 1999; Sahi and Alava 2003). A drop of batter was placed on the slide, in the central gap between the blades. Rhodamine B solution and Nile Red solution were added and the cover slide was carefully positioned to exclude air pockets. The images were observed and stored with a 1024 × 1024 pixel resolution using the microscope software (EZ-C1 v.3.40, Nikon).

2.4 Weight loss during baking

The weight loss during baking (WL) was calculated, taking into account the initial water content of each formulation (Rodríguez-García and others 2014b). The weight loss during baking was calculated as follows:

$$WL\ (g/100\ g) = [(B\text{-}C/IW) \times 100]$$

where:

WL= weight loss during baking

B = weight (in grams) of batter before baking.

C = weight (in grams) of cake after baking

IW = initial water content (in grams).

The initial water content was the sum of the initial water content of each ingredient in the formulation being measured. Measurements were performed in triplicate.

2.5 Cake height

The maximum cake height was measured on the vertical cross section of the product, using *ImageJ* software (National Institutes of Health, Bethesda, MD, USA).

The baked product was cut and photographed with a digital camera (E-510 Olympus, Hamburg, Germany). The images were stored in a 3648 × 2736 pixel format. The measurements were performed in triplicate.

2.6 Macroscopic structure of the crumb

The cakes were cut vertically in the central area and scanned using a HP Scanjet G2710 scanner (Hewlett-Packard, Palo Alto, CA, USA). The scanned images were analyzed using ImageJ software (National Institutes of Health, Bethseda, MD, USA). The image was cropped to a 5 × 5 cm section, on which the analysis was performed. Firstly, the image was split into color channels, then the contrast was enhanced and finally the image was binarized after applying a grayscale threshold. For each formulation, three different images were analyzed.

2.7 Color measurements

The instrumental measurements of the cake crust and crumb color were made with a Chroma meter CR-400 (Konica Minolta Sensing Americas, Inc., Ramsey, NJ, USA). The results were expressed in accordance with the CIELAB system, with reference to illuminant C and a visual angle of 2◦. The parameters determined were L^*(L^* = 0 [black], L^* = 100 [white]), a^* ($-a^*$ = greenness, $+a^*$ = redness), b^* ($-b^*$ = blueness, $+b^*$ = yellowness), C^*_{ab} (chroma [$C^*_{ab} = (a^{*2} + b^{*2})^{1/2}$]) and h_{ab} (hue [h_{ab} = arctan (b^*/a^*)]).

The total color difference (ΔE^*) was calculated as follows (Francis and Clydesdale 1975):

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

The values used to determine whether the total color difference was appreciable by the human eye were (Baixauli and others 2008):

$\Delta E^*<1$ color differences are not obvious to the human eye.

$1 < \Delta E^*<3$ color differences are not appreciated by the human eye.

$\Delta E^*>3$ color differences are obvious to the human eye.

The measurements were performed in quadruplicate.

2.8 Cake texture

Texture profile analysis (TPA) was carried out using a TA-TXT plus Texture Analyzer (Stable Microsystems, Godalming, U.K.) with Texture Exponent Lite 32 software (version 6.1.4.0, Stable Microsystems). The test was performed on 6 cubes (3 cm each side) taken from the central crumb of each cake. TPA was performed under the conditions described by Rodríguez-García and others (2012). The parameters studied were hardness, springiness, cohesiveness and chewiness. Measurements were performed in duplicate.

2.9 Sensory analysis

140 consumers (aged 18 to 64) were recruited among employees and students of the Universitat Politècnica de València. The samples were assessed in a standardized tasting room equipped with individual booths. Each consumer received 4 pieces of cake (1 piece each of the control with no OptiSol™5300 and the 3 fat-replacement cakes). 3-digit random numbers were used to code the pieces of cakes. They were served at room temperature in random order. Water was supplied to clean the consumers' mouths between each sample. Consumer acceptance testing was performed with a successive category scale to score the "appearance", "texture", "taste", and "overall acceptance" of the product. The scale was a 5-point

hedonic scale labeled 5 = like very much, 4 = like moderately, 3 = neither like nor dislike, 2 = dislike moderately and 1 = dislike very much.

2.10 Field Emission Scanning Electron Microscopy (FESEM)

Samples of each formulation (0.5 cm each side) were frozen at -80ºC and lyophilized (Telstar, Lyoquest 55, Terrassa, Spain). The samples were coated with platinum in a vacuum, then observed in a field emission scanning electron microscope (FESEM) (Zeiss, Ultra 55, Oberkochen, Germany).

2.11 *In vitro* digestion

The simulation of intestinal digestion consisted of a jacketed glass reactor (1 L capacity) maintained at 37 °C with continuous magnetic stirring at 120 rpm in a temperature-controlled circulating water bath throughout the test.

The *in vitro* digestions were carried out according to the method of Sozer and others (2014), with modifications. 4 g of ground sample, 100 mL 0.05 mol/L of sodium potassium phosphate buffer (pH 6.9) and 5 mL of 2.5g/100 mL pancreatin (P3292, pancreatin from porcine pancreas containing trypsin, amylase, lipase, ribonuclease and protease, Sigma-Aldrich, St. Louis, Mo., U.S.A) in 0.1 mol/L of pH 6 maleate buffer were placed in the jacketed glass reactor and maintained there for 120 min. Aliquots (7.5 mL) were removed at 20, 60, 90 and 120 min, placed in boiling water for 5 min, and cooled on ice. They were then centrifuged at 6600 rpm for 5 min and the supernatant was analyzed for reducing sugar content using the dinitrosalicylic acid (DNS) colorimetric method. The amount of digested starch was determined by multiplying the reducing sugar values by a 0.9 stoichiometric conversion constant for glucose to starch (Hardacre and others 2015). For each formulation, two different sponge cakes were analyzed.

2.12 Statistical analysis

Analysis of variance (ANOVA) was performed on the data using the Statgraphics Centurion XVI.I software package (StatPoint Technologies, Inc., Warrenton, VA, USA). Fisher's least significant difference (LSD) test was used to evaluate mean difference values ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1 Confocal laser scanning microscopy (CLSM) of the batters

Figure 1 shows CLSM images of the sponge cake batters prepared with different levels of fat substitution (R0, R30, R50 and R70).

In the control batter (R0), a continuous phase with a homogeneous appearance can be seen, stained red with rhodamine. It is mainly formed by interaction between the proteins of the sponge cake ingredients: milk, egg and gluten. Associated with this protein network, intact starch granules in black and fat globules stained green with Nile Red can also be identified. The fat globules are of different sizes, and many are clustered together, but hardly any coalescence can be seen.

In the R30 sponge cake batters, the continuous phase seems to be formed by interaction between the proteins of the different ingredients in the formulation (milk, egg, gluten and OptiSol™5300), and probably also the fat phase of the functional ingredient (OptiSol™5300).Unlike the sunflower oil, this fat phase does not appear in the form of globules but interacts with the protein fraction of the formulation. Another extensive dark or blackish network can be observed superimposed on the continuous phase. It is probably composed of the carbohydrate components of the OptiSol™5300 ingredient added to the formulation. The starch granules and fat globules can be seen associated with this network. Coalescence between the globules is slightly higher than in the control batter.

At the highest substitution level (R70), the extent, compaction and intensity of the dark/blackish network associated with the addition of OptiSol™5300 have increased. Also, as the levels of fat replacement in the sponge batter rise, fewer fat globules can be seen. Coalescence between the globules appears not to be significant in the high-substitution batters. The decreased fat content of these formulations appears to minimize coalescence.

3.2 Weight loss during baking and cake height

In general, adding OptiSol™5300 to the formulation led to significant low weight loss during baking ($p < 0.05$) from 21.48±0.51 g/100 g in the control (R0) to 20.09±0.36 g/100 g in R70 (Table 2). Hydrocolloids have the ability to increase moisture retention, although this depends on their chemical structure and interaction with the other ingredients (Gomez and others 2007), and consequently to reduce weight loss in the food. From this point of view, OptiSol™5300 presented hydrocolloid behavior in the sponge cake. The present results agree with those obtained by Khouryieh and others (2005), who observed lower moisture loss in muffins prepared with xanthan gum and maltodextrin as fat replacers than in those prepared with fat or with maltodextrin alone.

The addition of OptiSol™5300 to the formulation did not influence the maximum height of the sponge cakes. No significant differences ($p > 0.05$) between formulations were found in the values for this parameter (Table 2).

3.3 Macroscopic structure of the crumb

Figure 2 shows scanned, contrasted and binarized images of the different sponge cakes (R0, R30, R50 and R70).

The crumb macrostructure of the control cake (R0) was practically homogenous (Figure 2). In contrast, a series of diffusion pathways were observed in the crumb of the sponge cakes formulated with OptiSol™5300. Furthermore, these pathways appeared to a greater extent in the formulations with higher levels of substitution (R50 and R70) than in the R30 sponge cake.

3.4 Color measurement

The color data (L^*, a^*, b^*, C_{ab}^*, h_{ab}^*, ΔE^*) for the crust and crumb of the different sponge cakes studied are shown in Table 3.

In the crust, the values for parameters L^*, a^*, b^* and C^*_{ab} were significantly lower ($p < 0.05$) in the control formulation (R0) than in the fat substitution formulations studied (R30, R50 and R70), and no significant differences ($p > 0.05$) were observed between the formulations with the different levels of substitution. Lee and others (2004) also found significant differences in L^* for the crust of cakes in which fat had been replaced by oat bran and powdered flaxseed: the greater the replacement of fat by these ingredients, the higher the L^* values obtained, indicating greater luminosity. As regards hue (h_{ab}^*), significant differences ($p < 0.05$) were found between the control cake and the formulations with higher levels of substitution. Lastly, differences in overall color ($\Delta E^*>3$), visible to the human eye, were found between the control cake and the other formulations. The reason for these differences was that the surface color became less dark as more OptiSol™5300 was included in the formulation. Shearer and Davies (2005) also found overall color differences in muffins prepared with 5 g flaxseed flour/100g batter, but not in those prepared with 2 g of this flour/100g batter.

No significant differences in the crumb of the four types of sponge cake studied were found for L^*, b^* or C^*_{ab} (Table 3). However, the a^* value rose significantly ($p < 0.05$) as greater quantities of OptiSol™5300 were added to the formulation. Lee and others (2004) also encountered higher a^* values on increasing the level of flaxseed powder used to replace fat in cakes. In contrast, the h^*_{ab} value fell significantly as more OptiSol™5300 was added, as shown in Table 3. Lastly, no significant differences in overall color were encountered, as all the ΔE* values were below 3.

3.5 Sponge cake texture

Table 4 shows the parameters obtained from the texture profile analysis (TPA) curves.

The hardness values showed no significant differences ($p > 0.05$) between the control cake and R30. However, the addition of OptiSol™5300 in the formulations with higher fat replacement (R50 and R70) gave place to significant ($p < 0.05$) higher hardness values. Zambrano and others (2004) obtained a significantly higher firmness value in cake prepared with xanthan gum and 50% less fat than the control. Khouryieh and others (2005) also found significantly higher hardness values in muffins prepared with maltodextrin and xanthan gum to replace fat, when compared with the control.

The springiness and cohesiveness values indicate that there were no significant differences between the formulations studied. This could be because all the sponge cakes contained the same flour and, therefore, the same proportion of gluten proteins, which are responsible for the elasticity and strength of the batter.

The chewiness values followed a similar trend to those for hardness. No significant differences ($p > 0.05$) were observed between control cake and R30. In contrast significant differences were observed between R0 and cakes with higher quantity of OptiSol™5300 in their formulations (R50 and R70). R70 was the chewiest sponge cake – which could be related to a more compact cake – which is why it was necessary to apply more force compared to the other cakes to ready it for swallowing. Khouryieh and others (2005) also found significantly higher chewiness values than those of the control in muffins prepared with less fat and the addition of maltodextrin and xanthan gum.

3.6 Sensory analysis

The mean sensory acceptability values for the appearance, texture, taste and overall acceptance of the control cake and the sponge cakes prepared with different levels of fat replacement are shown in Figure 3.

The statistical analysis showed that there were no significant differences ($p > 0.05$) between the control cake (R0) and R30 for the attributes of texture, taste and overall acceptance. In the case of taste, R0 and R50 did not exhibit any significant differences either. Nevertheless, the appearance value was significantly lower ($p < 0.05$) for R30 than for the control.

For all the attributes studied, it was found that the sensory acceptability fell significantly as the quantity of OptiSol™5300 in the formulation rose, since significantly lower values were obtained on comparing R50 with R0 (except for the attribute of taste, as commented above) and R70 with R0. Of all the fat replacement formulations studied, R30 seemed to have the highest sensory acceptability.

3.7 Field emission scanning electron microscopy (FESEM)

Figure 4 shows the images obtained through field emission scanning electron microscopy (FESEM), allowing observation of the sponge cake microstructure.

The structure of the control cake (R0) can be seen to be made up of a well-developed hydrated protein network, mainly formed by the gluten from the flour, into which the rest of the ingredients have been incorporated. The gluten network contains the partially gelatinized starch granules and the oil has acted as a lubricant, creating a flexible continuous structure. The fat globules coalesced during baking and liberated fat, which was incorporated into the matrix, creating a film coating, as can be seen in Figure 4.

The R30 cake shows a more irregular microstructure, as the oil coating is less extensively distributed compared to R0. With the higher fat replacement levels (R50 and R70), it becomes more difficult it to find the oil coating in the images. It may also be seen that as greater quantities of OptiSol™5300 were added to in the formulation, this ingredient acted as a filler in the spaces within the protein network, making the sponge cake more compact. This information was correlated with the textural values, since the hardness and chewiness increased significantly, compared with the R0 and R30 cakes, as the OptiSol™5300 content rose.

3.8 *In vitro* digestion

Table 5 shows the proportion of starch digested *in vitro* for each of the sponge cakes studied.

The gelatinized starch fraction digested during the first 20 min of the digestion process is considered rapidly digestible starch (RDS) (Hardacre and others 2015). After subjecting the different cakes (R0, R30, R50 and R70) to 20 min *in vitro* digestion, no significant differences ($p > 0.05$) in RDS values were found between R0, R30 and R50 cakes; being RDS values in R70 significantly lower ($p < 0.05$). The digestibility of starch values significantly decreased ($p < 0.05$) in cakes with higher levels of substitution (R70) after 60, 90 and 120 min too. Adding fiber when processing foods is known to limit starch gelatinisation and mask the starch granules, increasing the proportion of resistant starch as observed by Hardacre et al., (2015). This could be also related to the field emission scanning electron microscope (FESEM) images, where the starch granules in the R70 cake matrix were observed to be very compact within the matrix, which would limit their physical accessibility to the digestive enzymes.

4. CONCLUSIONS

OptiSol™5300, a functional ingredient derived from flax seeds, can be used to replace fat in sponge cakes when formulating low fat products; as a natural product, it allows clean labeling avoiding E-numbers. When preparing fat reduced cakes using this ingredient it is found that sponge cakes with 30% fat replacement present good sensory acceptability to consumers. The hydrocolloid properties of OptiSol™5300 avoid moisture loss without affecting batter rise, and the hardness and crumb color of the cakes are similar than in control. Sponge cakes with 30% fat replacement by this functional ingredient could be elaborated by food industry as an appropriate strategy to reduce fat and calories in this bakery product.

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AUTHOR CONTRIBUTIONS

Cristina Eslava-Zomeño elaborated the cakes and carried out experimental work on physicochemical analysis and *in vitro* digestion determinations. Amparo Quiles collaborated in the *in vitro* digestion determinations and carried out the CLSM studies. Isabel Hernando supervised the work and carried out the FESEM studies.

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TABLES

Table 1. Composition of the formulations studied (g/100 g, wheat flour basis)

Ingredients	R0	R30	R50	R70
Wheat flour	100	100	100	100
Sugar	100	100	100	100
Egg yolk	27	27	27	27
Egg white	54	54	54	54
Skim milk	50	50	50	50
Water	0	15	25	35
OptiSol™5300	0	3	5	7
Sunflower oil	46	31.6	22	12.4
Sodium bicarbonate	4	4	4	4
Citric acid	3	3	3	3
Salt	1.5	1.5	1.5	1.5

R0: control sponge cake, R30: sponge cake with 30 g/100 g of fat replacement; R50: sponge cake with 50 g/100 g of fat replacement; R70: sponge cake with 70 g/100 g of fat replacement.

Table 2. Mean weight loss during baking and maximum height of the sponge cakes, by formulation**.**

Parameter	R0	R30	R50	R70
Weight loss (%)	21.48^{a} (0.51)	20.68^{b} (0.27)	19.78^{c} (0.23)	20.09^{bc} (0.36)
Height (cm)	10.06^{a} (0.79)	9.72^{a} (0.10)	9.43^{a} (0.71)	9.32^{a} (0.41)

Values in parentheses are the standard deviations. Means in the same row without a common letter are significantly different ($p < 0.05$) according to the LSD multiple range test (n=3); R0: control sponge cake, R30: sponge cake with 30 g/100 g of fat replacement; R50: sponge cake with 50 g/100 g of fat replacement; R70: sponge cake with 70 g/100 g of fat replacement.

Table 3. Mean values of sponge cake color parameters, by formulation

	Crust color				Crumb color			
Parameter	**R0**	**R30**	**R50**	**R70**	**R0**	**R30**	**R50**	**R70**
L*	40.05^{a} (1.12)	42.57^{b} (1.81)	43.51^{b} (1.90)	43.06^{b} (2.22)	66.86^{a} (1.45)	66.39^{a} (1.99)	66.47^{a} (0.83)	65.40^{a} (2.02)
a*	14.91^{a} (0.5)	15.35^{b} (0.37)	15.31^{b} (0.55)	15.59^{b} (0.52)	-2.23^{a} (0.20)	-1.11^{b} (0.24)	-0.60^{c} (0.16)	-0.20^{d} (0.12)
b*	24.95^{a} (2.18)	27.30^{b} (2.26)	28.00^{b} (2.29)	28.69^{b} (1.67)	22.97^{a} (1.61)	22.48^{a} (1.46)	23.06^{a} (1.25)	22.28^{a} (0.60)
C^*_{ab}	29.08^{a} (2.00)	31.34^{b} (1.92)	31.94^{b} (1.88)	32.66^{b} (1.42)	23.08^{a} (1.61)	22.50^{a} (1.46)	23.06^{a} (1.25)	22.29^{a} (0.60)
h^*_{ab}	59.02^{a} (2.01)	60.54^{ab}(2.26)	61.20^{b} (2.59)	61.42^{b} (1.79)	95.56^{a}(0.56)	92.85^{b} (0.63)	91.48^{c} (0.38)	90.52^{d}(0.30)
ΔE*		3.47	4.62	4.84		1.31	1.68	2.59

Values in parentheses are the standard deviations. Means in the same row without a common letter are significantly different ($p < 0.05$) according to the LSD multiple range test (n=12); R0: control sponge cake, R30: sponge cake with 30 g/100 g of fat replacement; R50: sponge cake with 50 g/100 g of fat replacement; R70: sponge cake with 70 g/100 g of fat replacement.

Table 4. Mean values of sponge cake texture properties, by formulation

Formulation	Hardness (N)	Springiness	Cohesiveness	Chewiness (N)
R0	5.35^{a}(0.71)	0.88^{a}(0.01)	0.73^{a}(0.00)	3.45^{a}(0.43)
R30	5.25^{a}(0.66)	0.89^{a}(0.01)	0.73^{a}(0.01)	3.42^{a}(0.39)
R50	7.84^{b}(1.39)	0.89^{a}(0.01)	0.72^{a}(0.01)	5.01^{b}(0.79)
R70	9.39^{c}(1.81)	0.89^{a}(0.01)	0.73^{a}(0.02)	6.07^{c}(0.99)

Values in parentheses are the standard deviations. Means in the same column without a common letter are significantly different ($p < 0.05$) according to the LSD multiple range test (n=12); R0: control sponge cake, R30: sponge cake with 30 g/100 g of fat replacement; R50: sponge cake with 50 g/100 g of fat replacement; R70: sponge cake with 70 g/100 g of fat replacement.

Table 5. Proportion of starch (g starch/100 g sponge cake) digested during *in vitro* digestion of sponge cakes.

Formulation	g starch/100g sponge cake			
	20 min	**60 min**	**90 min**	**120 min**
R0	46.06^{a}(0.36)	54.16^{a}(0.00)	56.46^{a}(1.09)	58.51^{a}(1.33)
R30	47.94^{a}(1.45)	57.32^{ab}(0.00)	58.17^{a}(0.72)	58.94^{a}(2.53)
R50	48.79^{a}(1.45)	58.43^{b}(2.05)	61.50^{b}(0.84)	61.92^{a}(0.48)
R70	40.51^{b}(1.81)	52.11^{c}(1.33)	54.16^{c}(1.09)	52.20^{b}(1.21)

Values in parentheses are the standard deviations. Means in the same column without a common letter are significantly different ($p < 0.05$) according to the LSD multiple range test (n=2); R0: control sponge cake, R30: sponge cake with 30 g/100 g of fat replacement; R50: sponge cake with 50 g/100 g of fat replacement; R70: sponge cake with 70 g/100 g of fat replacement.

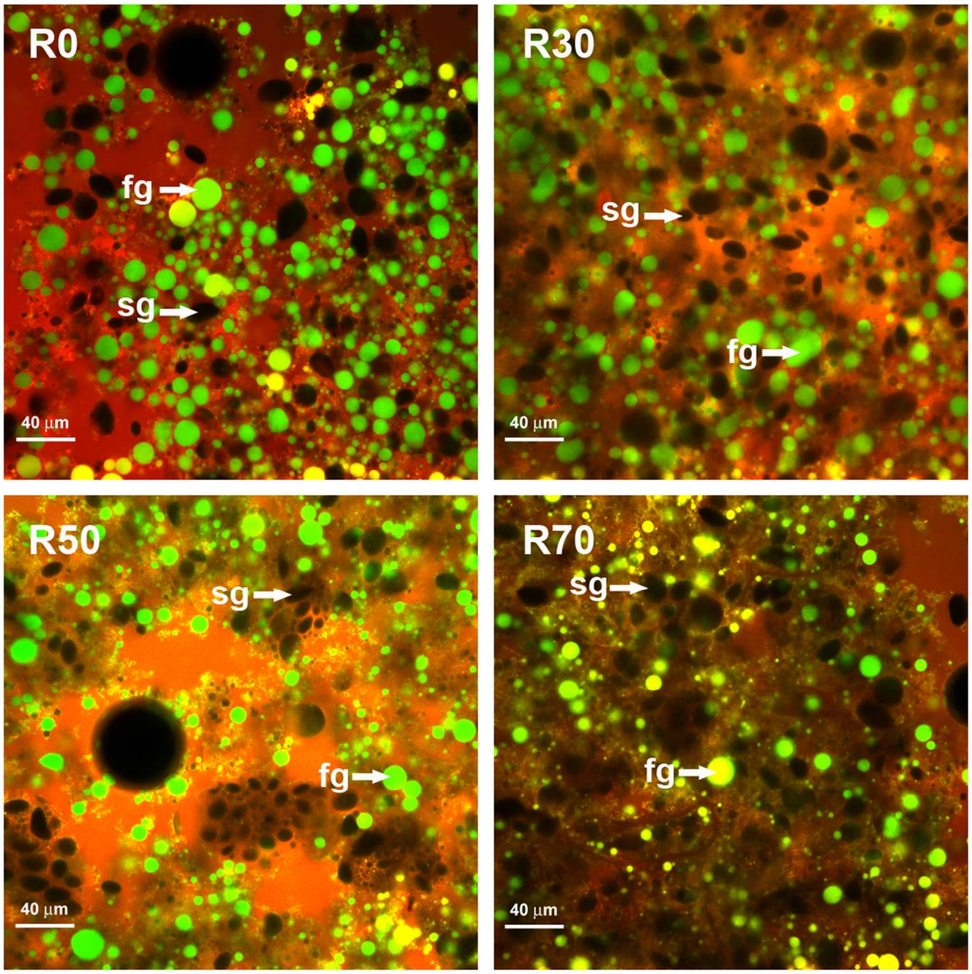
FIGURE CAPTIONS

Figure 1. Confocal laser scanning microscopy (CLSM) images of sponge cake batters R0, R30, R50 and R70. R0: control sponge cake, R30: sponge cake with 30 g/100g of fat replacement; R50: sponge cake with 50 g/100g of fat replacement; R70: sponge cake with 70 g/100 g of fat replacement. sg: starch granules, fg: fat globules. Magnification 40X.

Figure 2. Scanned images of whole R0, R30, R50 and R70 sponge cakes, 5 x 5 cm field, and respective binarized images. R0: control sponge cake, R30: sponge cake with 30 g/100g of fat replacement; R50: sponge cake with 50 g/100g of fat replacement; R70: sponge cake with 70 g/100 g of fat replacement. Arrows: diffusion pathways

Figure 3. Mean consumer acceptability values of the different sponge cakes. Means in the same attribute without a common letter are significantly different ($p<0.05$) according to the LSD multiple range test (n=140); R0: control sponge cake, R30: sponge cake with 30 g/100g of fat replacement; R50: sponge cake with 50 g/100g of fat replacement; R70: sponge cake with 70 g/100 g of fat replacement.

Figure 4. Field emission scanning electron microscopy (FESEM) images of R0, R30, R50 and R70 sponge cakes. R0: control sponge cake, R30: sponge cake with 30 g/100g of fat replacement; R50: sponge cake with 50 g/100g of fat replacement; R70: sponge cake with 70 g/100 g of fat replacement. ~~sg: starch granules,~~ o: oil. Magnification 200x. Bar = 20 µm.



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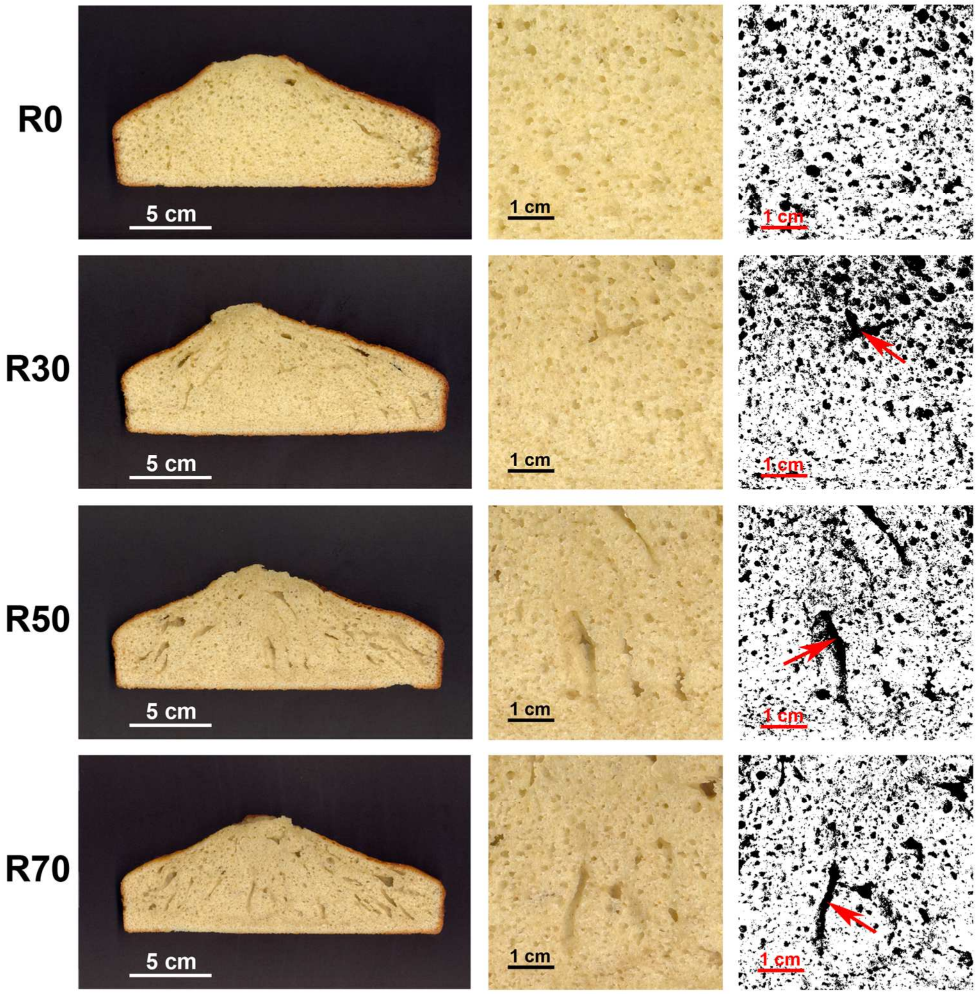

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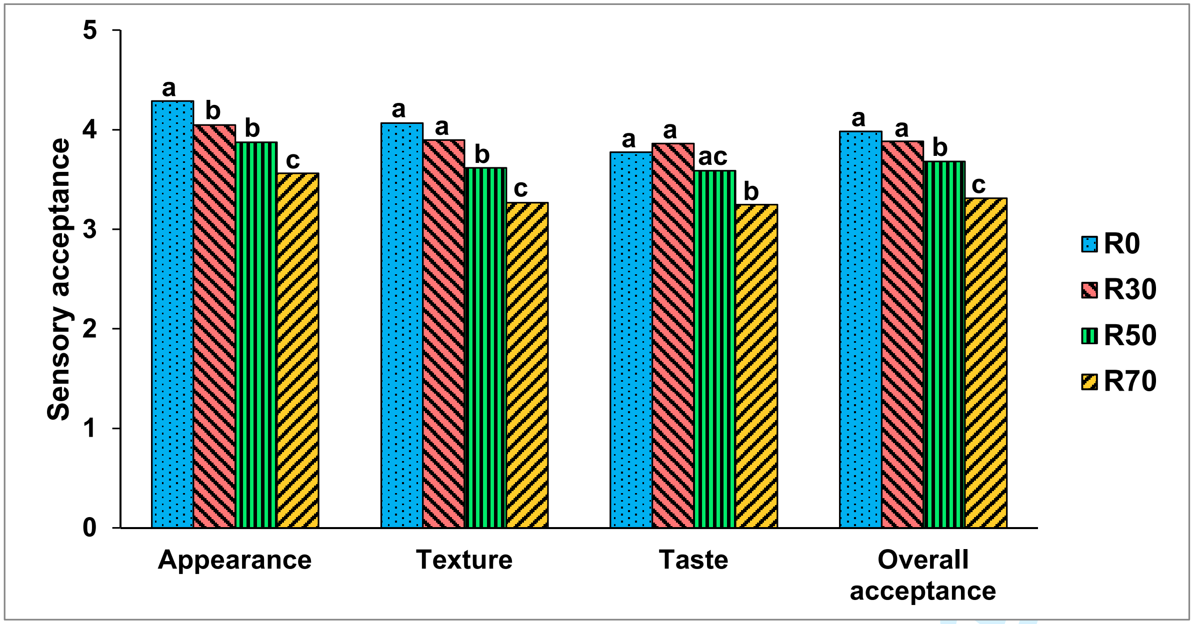
5
4
3
2
1
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Sensory acceptance
a
b
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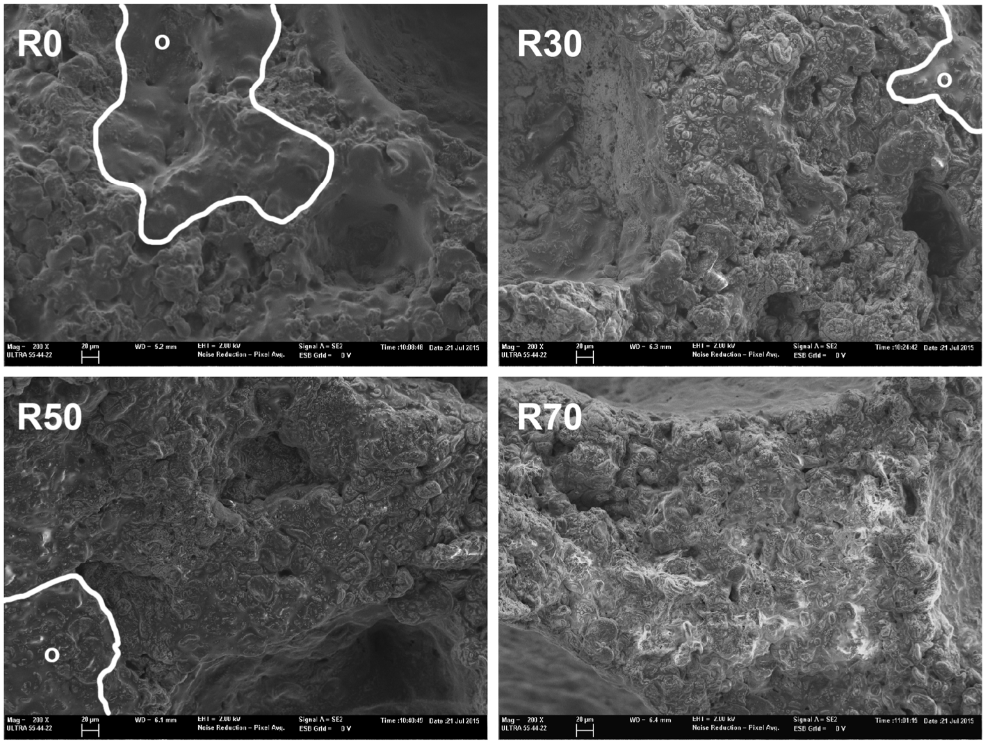
Appearance
Texture
Taste
Overall acceptance
R0
R30
R50
R70



155x117mm (300 x 300 DPI)

Answer to editor and reviewer

Comments to the Author:
Please address the comments of the reviewer and mine below in a separate cover letter and make all corrections in red font in the revised manuscript.

- Line 154 to 160 Why do you need to include IW in the equation? Why not just use the difference in weight before and after baking?

We decided to include IW in the equation because the initial content of water is very different as we need to add water depending of the amounts of Optisol used in the formulation. We have done it in this way in previous works as that published in Journal of Food Science:
Rodriguez-Garcia J, Puig A, Salvador A, Hernando I. 485 2012. Optimization of a sponge cake formulation with inulin as fat replacer: structure, physicochemical, and sensory properties. J Food Sci 77(2):C189-C97.

- Line 237 Why was amylase not included in the procedure?

The pancreatin used in this study includes amylase. The P3292 pancreatin (Sigma-Aldrich) contains:trypsin,amylase,lipase,ribonuclease and protease produced by exocrine cells of the porcine pancreas.
The composition of the pancreatin has been added to the manuscript (lines 240-241)

- Line 275 to 276 R50 seems to have less darkness than the other samples and the control. Please clarify statement.

It was a mistake. This sentence has been changed into "At the highest substitution level (R70) (line 278)"

- Line 325 The values decreased not rose for a* as more fiber was added. Please revise statements in this section as the reference found the opposite to what you found.

In the crumb, a* values increased from -2,23 in R0 to -0,20 in R70, as observed by Lee et al. (2004). In fact they also found negative values for a* when using low levels of substitution (e,g. 20% of flaxseed powder instead of shortening)

- line 359 to 364 The error bars on figure 3 overlap which means the samples and control are not different at all. Please carefully review the results and revise your statements.

It is true that the error bars in the figure overlap, which can lead to misunderstandings. In fact, in acceptability studies conducted with consumers the results are usually presented without error bars because acceptability can vary a lot among consumers. So, the statistics are often given only with the letters indicating the significant differences (or their absence). Some papers where the acceptability results are presented in this way are:

- *Rodríguez-García J. et al. 2012. Optimization of a sponge cake formulation with inulin as fat replacer: structure, physicochemical, and sensory properties. J Food Sci 77(2):C189-C97.*
- *Corral, S. et al. 2014 Effect of fat and salt reduction on the sensory quality of slow fermented sausages inoculated with Debaryomices Hansenii yeast. Food Control 45: 1-7*
- *Tarancón, A. et al. 2015. Use of healthier fats in biscuits (olive and sunflower oil): changing sensory features and their relation with consumers' liking. Food Research International 69 (2015) 91–96.*
- *laura laguna et al.2012. Balancing texture and other sensory features in reduced fat short-dough biscuits. Journal of Texture Studies 43: 235-245.*

- Figure 4 I cannot see the starch granules that are identified with arrows. Please clarify the figures or remove the identification of starch granules.

We agree to the editor. The starch granules are hard to distinguish. We have removed their identification in the figure (line 562).

- lines 395 to 399 Please explain why digestibility changed.

The explanation has been added to the manuscript (lines 402-408). "Adding fibre when processing foods is known to limit starch gelatinisation and mask the starch granules, increasing the proportion of resistant starch as observed by Hardacre et al., (2015). This could be also related to the field emission scanning electron microscope (FESEM) images, where the starch granules in

the R70 cake matrix were observed to be very compact within the matrix, which would limit their physical accessibility to the digestive enzymes."

Reviewers' Comments to the Author:
Reviewer: 1

Comments to the Author
- L98-108 L113, Table 1: A typical sponge cake is made based on the flour, sugar, and eggs. However, a oil was used in your cake preparation. Citation(s) should be mentioned with brief explanation for the oil usage in a sponge cake preparation.

The use of oil instead of shortening in products as sponge cakes and muffins is typical in Mediterranean countries. Some references where oil is used in these products have been added in the manuscript (lines 108-110). "Oil was used instead of shortening as usually done in Mediterranean countries (Rodriguez-Garcia and others, 2012; Martinez-Cervera and others 2011)." The explanation about how to use oil in sponge cakes preparation is provided in line 124-125 (the oil was finally placed on the top)

- L126: batter quantities for being placed in a pan should be mentioned. Total amounts of the prepared batters were different, due to the different amounts of the fat replacers. It may affect the optimum cake texture.

Yes, it is true. It may affect texture and other characteristics as height. We always use the same amount of batter (700 g) for each cake. It has been added to the manuscript (line 128) as we forgot to mention it in the previous version.

- L282-295: Data of weight loss and cake height should be presented.

Data have been included in Table 2

-L391-399 L408-410 Table 4.: The digestibility significantly increased or the same with the increased substitution levels of R30 or R50, but significantly decreased with the highest substitution levels of R70, in all the digestion periods. More explanation is needed for the sudden decrease with the samples with R70 citing more references.

An explanation had been included following the reviewer's suggestion (lines 402-408)

- L401-413: More discussions seem to be needed. Most of the results are jumping to the conclusions such as "clean-labeled ...". To me, sunflower oil is more natural than the processed OptiSolTM5300

This section has been rewritten trying not to list again the results and avoiding the jump to the last conclusion. The idea of preparing a low fat/low calorie product has been reinforced in this new version as sunflower oil is natural but more caloric than its replacer- Optisol+water (lines 411-419).