

Original article

## The role of lipid oxidation on biscuit texture during storage

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**Summary** The role of lipid oxidation and humidity absorption on the shelf life of biscuits was analysed. Biscuits with low lipid and sugar content were prepared employing different oils, and texture changes that biscuits experience during storage were studied. Water activity, peroxide value, malonaldehyde content and colour were also determined at different storage times. The maximum peroxide level was between 1 and 1.5 mmol kg<sup>-1</sup> of oil, at 166 days of storage. Values of malonaldehyde were between 5.2 and 15.8 nmol g<sup>-1</sup> of dry sample. Water activity and peroxide value influence the biscuit texture ( $P \leq 0.05$ ). To study the effects of both factors simultaneously, a mathematical model was adjusted to the data. Results indicate that an increase in the peroxide level, even at low level of lipid oxidation, increases Young's modulus and leads to a harder biscuit, while the humidity absorption lowers the fracture stress and Young's modulus.

**Keywords** Biscuit texture, lipid, lipid oxidation, mathematical model, storage time, water absorption.

### Introduction

Biscuits are a successful product in the food industry. They are easy to carry and store, but the great majority of biscuits are rich in sugar and fat, and they have sometimes been regarded as unhealthy foods (Conforti & Lupano, 2004). However, much research in recent years has focused on biscuit ingredients and proportions, which could be modified to achieve an alternative healthy product (Yamsaengsung *et al.*, 2012).

Biscuit texture parameters are important quality characteristics (Conforti *et al.*, 2012). Unfortunately, the texture of biscuits prepared with fat replacers or with low oil content is different from the conventional biscuits (Tarancón *et al.*, 2013). Hence, it would be a fundamental issue to have a complete evaluation of the factors that determine the biscuit texture, to control this important quality parameter. For the purpose of making healthier biscuits, we focused our work on biscuits prepared with low content of oil and sugar.

Different works have recently analysed the effect of composition and storage conditions on biscuits (Bhanger *et al.*, 2008; Pajin *et al.*, 2012). It is already known that water absorption may change the texture of biscuits (Kumar *et al.*, 2012), while oxidation may change their flavour (Mildner-Szkudlarz *et al.*, 2009). However, the effect of lipid oxidation on biscuit

texture remains unstudied. In this work, texture parameters, moisture content, water activity and peroxide value were determined during biscuit shelf life. Lipid oxidation can be retarded by lipid antioxidants such as flavonoids (Kiokias & Varzakas, 2014), as well as several natural vitamins and different herbal phenolic extracts (Kiokias *et al.*, 2008).

Mathematical techniques can be used to study the effects of many variables on texture deterioration of baked products. Previous works have developed predictive equations to describe the relationships between effects of storage conditions on texture of storage cakes (Gómez *et al.*, 2010). However, corresponding models for predicting textural changes in biscuits are not available.

In the present work, water activity and peroxide value were found to be important factors in determining the texture of biscuits. Therefore, a mathematical model was proposed to study the simultaneous effect of water activity and peroxide value on the texture of biscuits.

### Materials and methods

#### Materials

Biscuits were prepared with wheat flour (Favorita 000; Molinos Río de la Plata, Buenos Aires, Argentina), corn starch (Maizena, Unilever de Argentina S.A., Buenos Aires, Argentina), skim milk powder (SanCor;

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Sunchales, Santa Fe, Argentina), corn oil (Cocinero; Molinos Río de La Plata), sunflower oil (Cocinero; Molinos Río de La Plata), high oleic sunflower oil (Propia; Lezama, Buenos Aires, Argentina), sucrose (Ledesma, Jujuy, Argentina) and baking powder (Royal, Kraft Foods, Buenos Aires, Argentina).

All ingredients were of food grade. All chemicals used were of analytical grade.

### Biscuit preparation

Three formulations were prepared using corn, sunflower or high oleic sunflower oil. All formulations contained 70 g of flour, 20 g of skimmed milk powder, 60 g of corn starch, 25 g of sugar, 1.3 g of baking powder, 20 g of oil and 36.5 mL of tap water.

Solid ingredients were mixed in a kneading Philips Cucina mixer (HR 7633, Sao Paulo, Brazil) with 20 g of oil added in three fractions, mixing each time with three pulses (60 rpm) of 2 s. Water was added in two fractions mixing each time with fifteen pulses (60 rpm) of 2 s. Dough was put into a polyethylene bag and was manually kneaded until it becomes clay type dough. Then it was left at room temperature for at least 15 min before sheeting with a rolling pin to give it a thickness of 0.3 cm. The sheet was left to rest again for 15 min; to avoid desiccation, it was covered with a polypropylene film (Conforti & Lupano, 2004). Dough pieces were punctured to avoid big bubbles formation and cut to rectangular pieces with a pastry cutter (2.5 × 5 cm). Dough rectangles were placed on a silicon film and baked in an oven (F9M; Ariston, Fabriano, Italy) at 190 °C during 9.5 min. The oven temperature was registered by means of thermocouples.

Biscuits were vacuumed packed (Vacuumed Saver, Food Saver, Tila, Italia) in packages of five biscuits each and stored at 20 °C in polypropylene bags until analysis. To evaluate the effect of lipid oxidation and water absorption, the packaging material used for this study was permeable to oxygen and humidity. Samples were taken at different storage times during 6 month.

### Rancimat analysis

To evaluate the oxidation stability of the three oils used, a Rancimat analysis was performed following the method described by Issaoui *et al.* (2009) with slight modifications. About 5 g of sunflower, corn and high oleic sunflower oil was placed in a Rancimat 743 (Metrohm AG, Herisau, Switzerland). Samples were warmed at 110 °C with an air flow of 20 L h<sup>-1</sup>. Oil stability was expressed in terms of induction time (h).

### Lipid extraction

Lipids from milled biscuits (7.6 g) were extracted according to the method described by AACC (1983) for baked cereal products. To prevent lipid oxidation during the extraction procedure, subdued light was used and the sample heating was avoided (McClements & Decker, 2008).

Extracted lipids were used in peroxides determination.

### Peroxide determination

Peroxide value determination was performed in the oils extracted from biscuits according to the method described by the IDF-ISO (2006), using isopropyl alcohol as solvent. Absorbance was measured at 500 nm, using a UV-mini 1240 Spectrophotometers Shimadzu (Kyoto, Japan). A FeCl<sub>3</sub> solution (10 µg mL<sup>-1</sup>) was used for external calibration. The peroxide value was expressed as mmol of oxygen per kg of oil. All determinations were performed at least in duplicate.

### Malonaldehyde determination

The determination of malonaldehyde was performed on biscuit ethanolic extracts using the method described by Mišan *et al.* (2011). Results were expressed as nmol of malonaldehyde (MDA) per g of dry sample.

### Water activity (a<sub>w</sub>)

Water activity of biscuits was determined at 25 °C with an AquaLab Series 3 TE device (Decagon Devices, Pullman, Washington, DC, USA). Determinations were made at least in duplicate.

### Moisture

Biscuit samples were ground with a mortar. The moisture content was determined through weight loss after heating the sample in an oven at 105 °C until constant weight (AOAC, 1984). Determinations were made at least in duplicate.

### Superficial colour

Superficial colour was measured on three different zones of the top surface of biscuits with a Chromameter CR 300 Minolta (Osaka, Japan). The parameters Chroma (eqn 1), Browning Index (BI) (eqn 2) and Hue angle (eqn 3) were calculated from the Hunter L\*, a\* and b\* parameters (Bal *et al.*, 2011). Values are the average of at least four biscuits.

$$\text{Chroma} = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$\text{BI} = \frac{100(X - 0.31)}{0.17} \quad (2)$$

where

$$X = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.01b^*}$$

$$\text{Hue} = \tan^{-1}\left(\frac{a^*}{b^*}\right) \quad (3)$$

### Biscuit texture

Fracture properties of biscuits were studied by a three-point bending test, carried out at room temperature with a TA.XT2i texture analyzer (Stable Micro Systems, Godalming, UK). The width ( $d$ ) and thickness ( $b$ ) of biscuits were measured with a vernier calliper. Span length was 1.7 cm, and compression speed was set at  $0.5 \text{ mm s}^{-1}$ . The samples were placed on supports with their top surface down. The strength ( $F$ , Newton) needed to break the biscuits and the deformation ( $Y$ , cm) were obtained from the force-deformation curve (Baltsavias *et al.*, 1997). These parameters were used to calculate the fracture stress ( $\sigma$ ,  $\text{N cm}^{-2}$ ), fracture strain ( $\varepsilon$ ) and Young's modulus ( $E$ ,  $\text{N cm}^{-2}$ ) (eqns 4, 5 and 6 respectively).

$$\sigma = \frac{3FL}{2db^2} \quad (4)$$

$$\varepsilon = \frac{6bY}{L^2} \quad (5)$$

$$E = \frac{L^3s}{4db^3} \quad (6)$$

where  $L$  is the span length (1.7 cm) and  $s$  is the tangent of linear region of the curve force vs. deformation (Baltsavias *et al.*, 1997).

### Statistical analysis

The effects of oil type and storage time on biscuits were subjected to a two-way analysis of variance (ANOVA) at the 0.05 significance level. The least significant differences (LSD) were calculated to compare the means at a level of 95% ( $P \leq 0.05$ ) using the Fisher test (InfoStat, 2012; Córdoba, Universidad Nacional de Córdoba, Argentina).

Three-dimensional plots were drawn to reveal the combined effects of peroxide value and water activity on texture parameters. SYSTAT 12 software (SYSTAT, Inc., Evanston, IL, USA) was used in the computed procedure. The best model for each textural parameter was chosen by maximising the coefficient of determination

$R^2$  (Gómez *et al.*, 2010) preserving a significant  $F$ -ratio (Alvarez *et al.*, 2002). The coefficient  $R^2$  is a measurement of the degree of fitness; it measures how much variability in the observed response can be adjusted to the experimental factors (Cutright & Meza, 2007). To provide a better evaluation of the model adequacy, the parameter  $\text{adj-}R^2$  was also considered, as it adjusts the number of terms in the model (Battaiotto *et al.*, 2013; Ferrari *et al.*, 2013). To evaluate the failure of the proposed model to represent data in the experimental domain at which points that were not included in the regression (Varnalis *et al.*, 2004), the lack of fit test was performed. A nonsignificant lack of fit value indicates that the fitted model is appropriate for the description of the response surface (Boyaci *et al.*, 2004).

## Results and discussion

### Accelerated lipid oxidation assay (Rancimat)

The aim of the present work was to analyse the hydroperoxide content effect on biscuits during storage. To provide a way of reaching different oxidation levels at various storage times, oils that differ in their oxidation stability were considered to be the most appropriate for the biscuit formulations. Significant differences were found among the oxidation stability of the oils used in biscuits (Table 1). The values obtained indicate that the sunflower oil was the most susceptible to oxidation, whereas the most stable one was the high oleic sunflower oil ( $P \leq 0.05$ ).

### Lipid oxidation

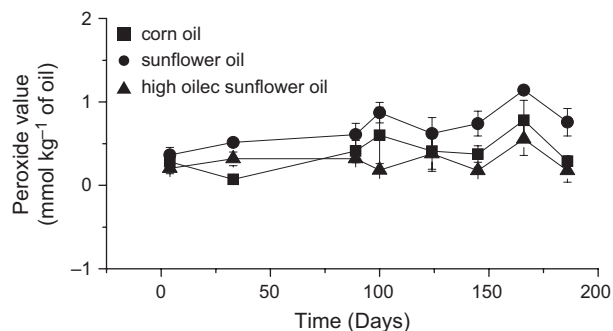
The extent of lipid oxidation of the oils extracted from biscuits was determined by the peroxide and malonaldehyde content.

The peroxide value of the three oils extracted from biscuits during storage was significantly different ( $P \leq 0.05$ ). The sunflower oil presented the highest peroxide content, followed by the corn oil, in agreement with Rancimat results. The maximum peroxide level was found at 166 days of storage (Fig. 1). The values obtained were similar to those found in processed cheese during storage (Kristensen *et al.*, 2001).

**Table 1** Rancimat induction times of different oils at 110 °C

	Induction time (hours)
Corn oil	11.4 <sup>b</sup>
Sunflower oil	2.9 <sup>c</sup>
High oleic sunflower	27.3 <sup>a</sup>

Values with a common letter do not differ significantly at the 5% significance level.



**Figure 1** Peroxide value of biscuits prepared with corn oil (■), sunflower oil (●) and high oleic sunflower oil (▲) vs. time. Bars show standard deviation.

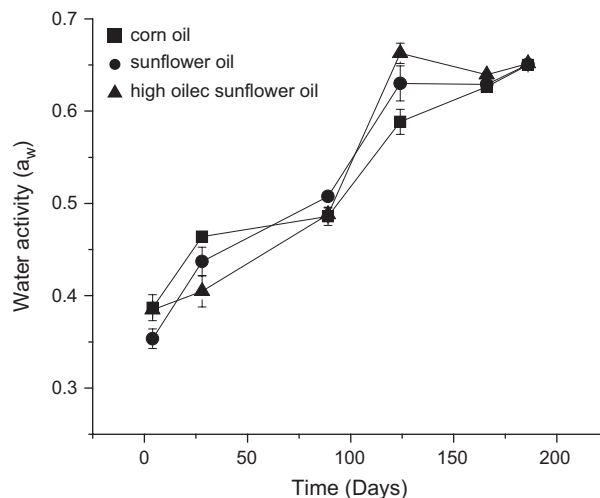
Sunflower oil contained the highest level of polyunsaturated fatty acids between the oils tested (Sabudak, 2007; Kiokias *et al.*, 2009); thus, it was reasonable that it presented the main oxidative degradation. These results agree with the findings of Caponio *et al.* (2009) who studied biscuits prepared with margarines, which differ in their fatty acid composition; the highest oxidation level was observed in biscuits that contained high amounts of unsaturated fatty acids.

The malonaldehyde is a final product of lipid oxidation. Its content was determined at 145 and 186 days of storage, that is, before and after the maximum of peroxides. Values of nmol of malonaldehyde per g of dry sample were between 5.2 and 15.8. No significant differences were found between all the samples assayed ( $P > 0.05$ ). Mišan *et al.* (2011) established that  $< 20$  nmol of MDA per g of sample is considered to be a low quantity of this secondary product. According to the peroxide and MDA values obtained, biscuits analysed in the present work presented a low level of lipid oxidation.

#### Moisture content and water activity ( $a_w$ )

The moisture content of biscuits increased during storage at 20 °C ( $P \leq 0.05$ ). Biscuits exhibit moisture contents lower than 8.5% (wet basis) until 89 days of storage. From 89 days to the end of the study, moisture increased from 8.5 to 10.5%.

Biscuits absorbed humidity during storage, and, consequently, their water activity increased ( $P \leq 0.05$ ). The equilibrium was reached after 124 days with a water activity of 0.65. No significant differences were found between biscuits prepared with different oils during the length of the experiment ( $P > 0.05$ ) (Fig. 2). Previous works have indicated that the increase in moisture content could be explained by the hygroscopic nature of biscuits, storage conditions and the packaging material (Nagi *et al.*, 2012).



**Figure 2** Water activity of biscuits prepared with corn oil (■), sunflower oil (●) and high oleic sunflower oil (▲) vs. time. Bars show standard deviation.

Water absorption was probably the main limiting factor of biscuit shelf life. The water activity is a very important parameter to evaluate the microbiological stability of the product (Huchet *et al.*, 2013). The maximum of 0.65 reached in the present work is low enough to avoid microbial growth. However, the acceptability of the product may change. According to Hough *et al.* (2001), the crunchiness of biscuits decreases sharply when the water activity increases from 0.4 to 0.6.

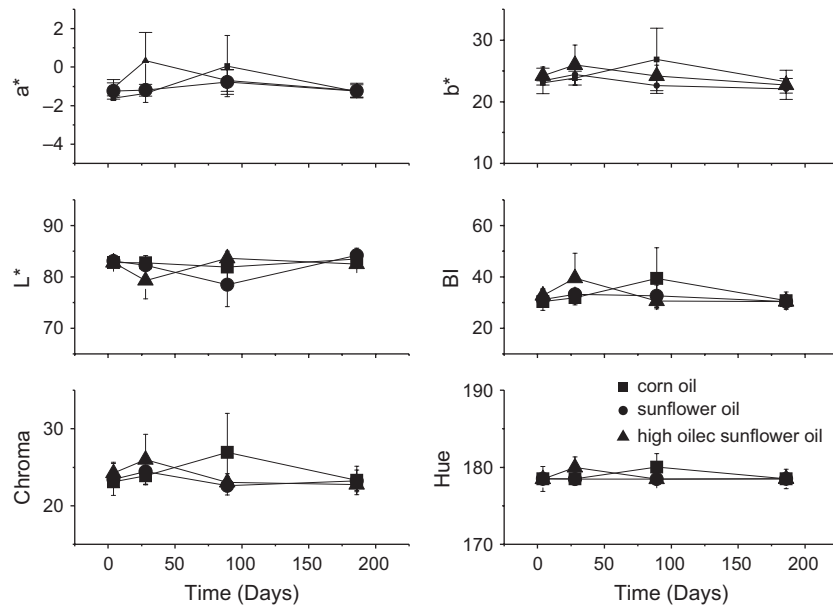
#### Colour

As it was previously mentioned, biscuits experience lipid oxidation and water absorption during storage. Free radicals produced during lipid oxidation could react with biscuit proteins modifying the product colour (Zamora & Hidalgo, 2005).

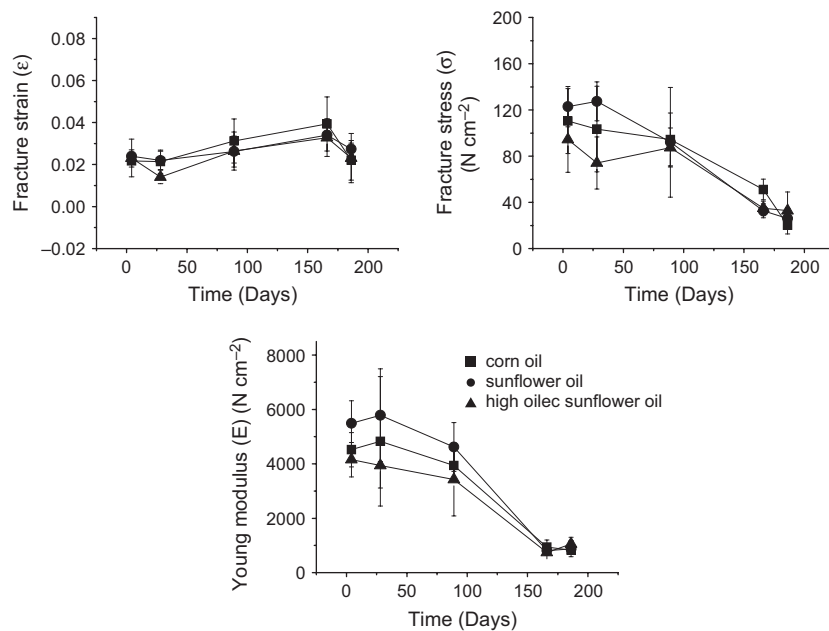
In the conditions tested, the biscuit colour remained unchanged during storage ( $P > 0.05$ ) (Fig. 3). No significant differences in the colour parameters ( $a^*$ ,  $b^*$ ,  $L^*$ , chroma, Hue or browning index) were found between biscuits prepared with different oils ( $P > 0.05$ ). This result could be attributed to the low lipid amount that the prepared biscuits have. Similar results were found by Hozova *et al.* (1997) in sensory evaluation of stored amaranth crackers.

#### Texture analysis

Figure 4 shows the results of biscuit texture analysis. Significant differences were observed in all texture parameters during biscuit storage ( $P \leq 0.05$ ). Data show that after 100 days of storage, fracture stress and



**Figure 3** Colour parameters of biscuits prepared with corn oil (■), sunflower oil (●) and high oleic sunflower oil (▲) vs. time. Bars show standard deviation.



**Figure 4** Texture parameters of biscuits prepared with corn oil (■), sunflower oil (●) and high oleic sunflower oil (▲) vs. time. Bars show standard deviation.

Young's modulus decreased, indicating that biscuits became more fragile and more susceptible to the fracture.

Biscuits prepared with different oils did not show any difference in the fracture strain ( $P > 0.05$ ), but presented significant differences in the fracture stress and Young's modulus ( $P \leq 0.05$ ). Biscuits prepared with sunflower oil were the hardest ones and exhibit the highest Young's modulus value.

In biscuits with similar values of water activity, a positive linear correlation was found between the peroxide value of the lipid fraction extracted from biscuits and the fracture stress of the biscuits ( $R^2 = 0.86$ ). The same tendency was found between Young's modulus and the peroxide value ( $R^2 = 0.99$ ) of the oils extracted from biscuits. This result seems to indicate that texture differences among biscuits were related to the peroxide value of the lipid fraction during storage.



**Table 2** Regression coefficients of the polynomial model

Coefficients	Fracture stress (N m <sup>-2</sup> 10 <sup>4</sup> )	Young's modulus (N m <sup>-2</sup> 10 <sup>4</sup> )
β <sub>0</sub>	-91.60	6523.73
β <sub>1</sub>	977.17	-7933.28
β <sub>2</sub>	9.39	12011.87
β <sub>12</sub>	NS	-20008.02
β <sub>11</sub>	-1228.70	NS
Model ( <i>F</i> -ratio)	38.06 <sup>a</sup>	51.98 <sup>a</sup>
<i>R</i>	0.82	0.88
<i>R</i> <sup>2</sup>	0.68	0.77
<i>R</i> <sup>2</sup> (adjusted)	0.66	0.75
Lack of fit ( <i>P</i> -value)	0.06 <sup>b</sup>	0.06 <sup>b</sup>

NS, not significant.

<sup>a</sup>Significant at a *P* < 0.001 level.

<sup>b</sup>Want the selected model to have nonsignificant lack of fit (*P* > 0.05).

Consistent with these results, previous authors have indicated the effect of lipid oxidation in the food texture (Zamora & Hidalgo, 2005). It is widely accepted that the oxidised lipids could react with proteins by cross-linking reactions modifying the product texture and functionality (Eriksson, 1982; Estévez *et al.*, 2005). Moreover, in agreement with our results, Toyosaki (2007) studied the effect of hydroperoxides on dough fermentation and found that oxidised linoleic acid could interact with gluten proteins inducing gluten denaturation, resulting in a tighten network.

In the present work, as results indicate that biscuits' texture depended not only on water activity, but also on biscuit peroxide value, both parameters were studied simultaneously. A mathematical model was adjusted to the data. To check the significance of each term, the *p*-values were calculated. Only significant terms were considered in the model (*P* ≤ 0.05).

The fracture stress showed that linear and quadratic terms were significant (*P* ≤ 0.05). Therefore, the following model was proposed

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 \quad (7)$$

where *X*<sub>1</sub> is the water activity, and *X*<sub>2</sub> is the peroxide value (mmol of oxygen per kg of oil), β<sub>0</sub> is a constant, β<sub>1</sub> and β<sub>2</sub> are the parameters estimated for linear terms and β<sub>11</sub> is the quadratic regression term.

Young's modulus showed that linear and interaction terms were significant (*P* ≤ 0.05). Thus, the following model was proposed, where β<sub>12</sub> is the interaction term.

$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_2 X_1 \quad (8)$$

Table 2 shows the model fitted to express the variation of texture parameters with water activity and peroxide value. Coefficient *R*<sup>2</sup> and adj-*R*<sup>2</sup> were good for fracture stress and for Young's modulus, considering that biscuits could present unavoidable texture

variations among them (Baltsavias *et al.*, 1997). The *R*<sup>2</sup> and adj-*R*<sup>2</sup> found for fracture stress were lower than 0.75, so that this model should only be used to analyse trends, not to predict, according to Alvarez *et al.* (2002). The *F*-ratios obtained for the models presented highly significant probability values (*P* < 0.001), indicating that the selected model adequately represented texture analysis data. In addition, in both cases, the parameter lack of fit was not significant (*P* > 0.05). Therefore, it could be assumed that the fitted models were appropriate for the description of the data.

The full models were used to generate the graphs of fracture strain and Young's modulus as a function of the sample peroxide value and water activity. These graphs indicate that an increase in the level of peroxides increases Young's modulus and leads to a harder biscuit, while the absorption of humidity lowers the fracture stress and Young's modulus.

## Conclusions

The presence of hydroperoxides, even at a low level, could increase biscuit hardness and Young's modulus, while it does not modify biscuit colour in low lipid biscuits. On the other hand, the increase in water activity is a very important factor in the biscuit texture, making the product easier to break.

Changes in texture parameters during storage of biscuits could be adjusted to a simple mathematical model, which contributes to a better understanding of textural changes in stored biscuits under controlled conditions. This model would help the industry and those wishing to develop a high-quality product by controlling the texture parameters. However, this is just a preliminary attempt to understand all the complex interactions between biscuit components that determine the texture of this bakery product.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** (a) Three-dimensional plot of fracture stress, water activity and peroxide value in biscuits. (b) Three-dimensional plot of Young's modulus, peroxide value and water activity in biscuits.