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Gluten-free wafer formulation: Development, characterisation and addition of flavourings with antioxidant capacity

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Abstract

The incidence of celiac disease is increasing, therefore the demand for gluten-free products that also satisfy the nutritional requirements of celiac individuals is rising. Thus, the objective of the present work was to develop a gluten-free nutritionally balanced wafer formulation with a high content of antioxidants. First, the animal fat used in the traditional formulation was successfully replaced by high oleic sunflower. Second, the antioxidant content of several flavourings (cinnamon/honey/anise/vanilla) was measured and their addition to a gluten-free wafer formulation was evaluated. Third, multivariate statistical tools were used to select the formulation that properly mimicked the characteristics of a gluten-containing wafer. According to the results, anise and cinnamon were the most suitable flavourings to prepare gluten-free wafers, and the sensory analysis concluded that these formulations were highly acceptable (means>6.7 on the hedonic scale). Finally, the storage time analysis indicated that the texture of the gluten-free wafers was more susceptible to water absorption than gluten-containing wafers. Besides, cinnamon wafers presented a higher bioaccessible antioxidant capacity than anise wafers (43.5 ± 0.1 mg Trolox/g and 18.8 ± 0.9 mg Trolox/g respectively) ($p < 0.05$), which remained stable for four months. This indicates that during its shelf life, the product could be consumed with its beneficial effects intact.

Keywords

Celiac disease, bioaccessible antioxidants, cinnamon, anise, shelf life

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INTRODUCTION

Celiac disease (CD) is an autoimmune enteropathy characterised by a persistent intolerance to gluten proteins (storage proteins contain wheat, rye, barley and probably oats) (Balakireva and Zamyatnin, 2016). This disorder affects the small intestine of genetically predisposed people in whom the consumption of gluten leads to a damage in the mucosa. This results in impaired absorption and, consequently, in multisystemic complications such as anaemia, rickets, osteoporosis, late development, dermatologic manifestations and short stature (Jnawali et al., 2016; Regula et al., 2018). It has been reported that inflammation and oxidative imbalance are involved in the mechanisms of CD, therefore the intake of some nutrients such as antioxidant vitamins (vitamin C and E), polyphenols and carotenoids may represent a useful approach for nutritional intervention in celiac patients (Dias et al., 2021).

To date, the only treatment is a strict gluten-free (GF) diet that must be followed throughout life. This enables the intestine to heal and the symptoms to resolve (Jnawali et al., 2016). GF products currently available in the market have a low nutritional quality: they present a lower content of proteins,

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fibre, minerals and vitamins than similar gluten products, while their fat and salt content is often higher (Jnawali et al., 2016; Melini and Melini, 2019). Therefore, in order to ensure an adequate intake of nutrients it is important to follow a varied diet. Larretxi et al. (2019) reported that a diet based on GF products increases the imbalance in the energy and nutrient intake of CD patients. This indicated the importance of considering other products from different food groups and not only exclude the prohibited gluten-containing food or replace it with GF equivalents. Di Nardo et al. (2019) recommended that naturally GF products should be incorporated in the diet of CD patients; however, because of the possibility of external gluten contamination during the technological processing, their gluten content should also be checked to assure its adequacy. Therefore, it would be necessary to conduct an analytical test of gluten content in food for particular uses and also in generally available foods to diversify the GF diet.

Besides, GF biscuits show several nutritional deficiencies. They often have a high sugar content, low protein amount and a high proportion of saturated lipids (Melini and Melini, 2019). Maggio and Orecchio (2018) analysed different bakery products for celiac people and indicated that GF wafers contained the highest amount of saturated fatty acids (72%). Therefore, the demand for acceptable healthy and nutritionally balanced products that not only guarantee the absence of gluten but also satisfy the requirements of individuals with CD or other gluten intolerance is a major issue in food development.

Moreover, in the last few decades the incorporation of antioxidants into the diet has been a matter of great concern. These bioactive compounds, which can reduce the level of lipid oxidation, have been associated with the protection against noncommunicable diseases (Grosso, 2018) and could have a beneficial effect on CD, as previously explained. Hence, their addition to the formulation of GF food is mandatory. Several flavourings are well known for their antioxidant properties, and their presence in sweet bakery products may have a double benefit: they would impart pleasant organoleptic characteristics while increasing the level of bioactive compounds.

Therefore, the objective of the present work was to develop a GF nutritionally balanced wafer formulation with a high content of antioxidants. In order to do this, the replacement of animal fat was evaluated, as well as the addition of different flavourings with antioxidant capacity. Finally, the sensory acceptability and the shelf life of the developed product were evaluated to assure that it will be well accepted in the market.

MATERIALS AND METHODS

A graphic diagram of the laboratory analyses carried out and the materials used can be found in the Supplementary Information.

Extraction of bioactive components from flavourings

Anise and cinnamon (1.5 g) were extracted with 10 mL of warm deionised water (45°C) according to Masutti et al. (2020). After 30 min of stirring (600 rpm) at ambient conditions (Dragon Laboratory (DLAB) HCM100-Pro, Beijing, China), the samples were centrifuged (10 min, 3500 × g). Honey and vanilla essence were weighed (10 g) and dissolved in water (final volume, 50 mL or 10 mL respectively). These solutions were saved and stored at − 20°C until analysis.

For the determination of the antioxidant capacity, three different methodologies described in the Supplementary Information were performed.

Wafer preparation

The GF wafer formulations were prepared as follows: 77.5 mL of water or the flavouring extract (anise, vanilla or cinnamon) prepared as previously explained was mixed manually with 5.85 g of the lipidic fraction (animal fat or its replacement with sunflower oil at 0%, 50% or 100%) and 13.5 g of the egg yolk for 1 min. Then, the dry ingredients were added (50.0 g of whole grain rice flour, 40.5 g of powdered sugar, 5.8 g of dried egg white, 1.0 g of salt and 0.1 g of xanthan gum) and mixed in a kneading Philips Cucina mixer (São Paulo, Brazil) for 3 min at 858 rpm, as recommended by Daniel and Dodd (2009). Besides, in order to properly replace sugar with honey (at 0%, 50% and 100%) in the GF honey wafers, the moisture content of this bee product was considered and evaluated by the refractometric method. The final composition of GF wafers was 75.5 g of carbohydrates; 10 g of proteins; 9.74 g of lipids; 1.9 g of dietary fibre; 1.0 g of minerals and 2 of water per 100 g of wafer.

For the wheat flour formulations, 12.50 g of butter was mixed manually with 105.0 g of water. Then, 75.0 g of wheat flour and 50.0 g of sugar were added and mixed in a kneading Philips Cucina mixer (3 min at 858 rpm).

For the cooking procedure, 10 mL of each mixture was placed in a waffle iron machine (Yanis, Villa Lynch, Buenos Aires, Argentina) at 200°C for 30 s. After that time, wafers were cooled at room temperature and analysed (Nasabi et al., 2021).

Water activity and moisture content of wafers

The water activity of the wafers was measured with a water activity metre (AquaLab Series 3, Decagon Devices, Pullman, USA) at 25°C, according to Azmoon et al. (2021). The moisture content was determined through weight loss after heating the sample in an oven at 105 °C until constant weight.

Colour and texture parameters

The CIELab scale was used to determine the colour (L*, a* and b* parameters) of the wafers according to Kouhsari et al. (2022) with slight modifications. All the determinations were performed with a Minolta Chromameter CR 300 (Osaka, Japan). The total colour difference (ΔE) between the wheat wafer (L_0^*; a_0^*; b_0^* used as control) and the GF wafers was determined according to the following equation:

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

The diameter and the thickness of the wafers were measured with a calliper. The texture of the products was studied by a three-point bending test run at room temperature with a TA.XT2i texture analyser (Stable Micro Systems, Godalming, UK). The fracture stress (σ, Pa = N/m^2) and the fracture strain (ϵ) were determined according to the equations developed by Mohammed et al. (2013).

Sensory analysis

Ninety-six consumers (40% men and 60% female) between 18 and 66 years of age were recruited at the National University of La Plata. All the participants were informed about the general aim of the study and gave their consent prior to their participation. The samples were coded and supplied to the consumers who rated their "colour", "texture", "flavour" and "overall acceptance" on a hedonic scale (Baixauli et al., 2008). The panellists also indicated in the survey sheet if they usually consumed GF products and their acceptability to anise and cinnamon taste.

Shelf-life analysis

Wafers were prepared and stored at 20°C in sealed polypropylene bags for 4 months. At different storage times the physical parameters were evaluated, as well as the antioxidant capacity after an *in vitro* digestion procedure, as described in the Supplementary Information.

Statistical analysis

An analysis of variance (ANOVA) followed by a Fisher's test was used to statistically analyse the results. In both cases, a 0.05 significance level was used (InfoStat, 2012; UNC, Argentina). In order to evaluate the strength of the relations between variables, Pearson's correlation coefficients were calculated. Besides, two multivariate statistical analyses were performed: Principal component analysis (PCA) and hierarchical cluster analysis (Balzarini et al., 2008).

RESULTS AND DISCUSSION

Antioxidant capacity of usually consumed flavourings

Cinnamon presented the highest content of phenolic compounds (263.86 ± 20.13 mg GAE/g), while honey presented the lowest values (0.43 ± 0.05 mg GAE/g) ($p \leq 0.05$) (Table 1). The total phenolic compounds in cinnamon were similar to the ones found by Mathew and Abraham (2006), while the phenolic compounds in honey was in line with results by Gozález-Ceballos et al. (2021) in Spanish honeys. Honey is mainly composed of simple sugars (glucose and fructose) and may present different organic acids, flavonoids and phenolics, which contribute to its antioxidant effect (Erejuwa et al., 2012). However, according to the present results, these compounds are in a low concentration compared to the other flavourings analysed.

In the same line, the ferric reducing ability (FRAP) assay showed that cinnamon presented the highest antioxidant capacity, followed by vanilla, anise and finally honey. Moreover, good correlations were found between the total phenolic compounds and the FRAP antioxidant activity (r > 0.9). Because both assays measure the reducing capacity, previous researchers have already indicated good associations between these assays in different food products (Wootton-Beard et al., 2011). This seems to indicate that the antioxidant capacity determined by FRAP assay in the flavourings accounts for the presence of phenolic compounds in the samples.

According to the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay, the antioxidant capacity increased in the following order: honey<cinnamon<anise<vanilla ($p \leq 0.0.5$). A similar order was found in the antioxidant activity measured with 1,1-diphenyl-2-picrylhydrazyl (DPPH), but no significant differences were found between cinnamon and anise or between cinnamon and

Table 1. Antioxidant capacity and FC reactive substances of honey, anise, vanilla and cinnamon according to FRAP, DPPH, Folin, ABTS expressed as dry matter.

	Honey	Anise	Vanilla	Cinnamon
Folin(mg GAE/g)x	0.430 ± 0.050^a	47.36 ± 0.46^b	208.61 ± 0.35^c	263.86 ± 20.13^d
ABTS(mg TE/g)	0.002 ± 0.002^a	6.78 ± 0.01^c	10.11 ± 0.07^d	3.21 ± 0.06^b
DPPH(mg TE/g)	0.003 ± 0.001^a	0.02 ± 0.01^b	0.07 ± 0.02^c	0.01 ± 0.003^{ab}
FRAP(mg TE/g)	0.005 ± 0.003^a	1.05 ± 0.03^b	2.07 ± 0.07^c	4.65 ± 0.71^d

*Results are expressed as mean ± standard deviation. Different letters within each column indicate significant differences among samples according to Fisher's test (p ≤ 0.05).

honey ($p > 0.05$). The statistical analysis showed a strong correlation between both assays (r > 0.97). ABTS and DPPH assays evaluate the ability of a sample to neutralise the free radicals, and other authors have also demonstrated important correlations between them, which indicates that both methods could be equally useful for assessing the antioxidant capacity in natural extracts (Awika et al., 2003).

Generally speaking, honey was the ingredient with the lowest antioxidant capacity, while vanilla and cinnamon showed the highest values. The high content of phenolic compounds found in vanilla may be due to the presence of ethyl vanillin (Tai et al., 2011). On the other hand, compounds responsible for the antioxidant effect of cinnamon include cinnamic acid, cinnamaldehyde, cinnamate and eugenol, which counteract the damaging effects of free radicals and can protect against mutagenesis (Sánchez, 2013).

Finally, anise showed an intermediate antioxidant capacity. Studies performed by Quilca (2011) on the composition of essential oils of this flavouring revealed the presence of high amounts of anethol and its isomers, which are associated with their antioxidant properties.

Development of GF wafers: selection of the lipidic fraction

According to the results provided in the Supplementary Information, it was possible to fully replace the fat generally used in the wafer formulations with high oleic sunflower oil and obtain a product with appropriate characteristics for the consumers who expect a crisp and dry texture.

Addition of flavourings with antioxidant capacity to GF wafers

The characteristics of seven different wafer formulations were analysed: the original GF formulation without flavourings (Or-GF); two honey formulations with different levels of sugar replacement (100% or 50%, GF-H100 and GF-H50 respectively); a formulation with anise extract (GF-Anise); a formulation with cinnamon extract (GF-Cinnamon) and a formulation with vanilla extract (GF-Vanilla) addition. Besides, a formulation prepared with wheat flour (wheat formulation) was also included in the assays.

Water activity and moisture content. As detailed in Table 2, the presence of honey significantly increased the moisture content of the wafers ($p \leq 0.05$). This is probably explained by its high proportion of glucose and fructose, which are more hygroscopic than the sucrose used in the other formulations. Similar results were obtained by Adeboye and Bamgbose (2015), who support the idea that the addition of honey to the formulations of biscuits increased their moisture content, because it reduces moisture diffusion in the batter during baking. On the other hand, the lowest moisture level was found in the wheat flour formulation ($p \leq 0.05$). This may be associated with the high-water absorption of rice flour-based dough used in the GF products: according to Mancebo et al. (2015), the water binding capacity of rice flours could be up to 58% higher than that of wheat.

In spite of the differences observed in the moisture, no significant differences were found among the a_w of the assayed formulations ($p > 0.05$). This seems to indicate that water remains strongly bound and only partially available in the wafer structure because of its strong association with the food components in the final matrix.

Moreover, all the wafer formulations presented an a_w value below 0.5, therefore it could be considered that the microbial deterioration would be inhibited. Most spoiling bacterial growth ceases at an a_w below ≈0.90, while mycotoxigenic moulds have a limit for growth at a ≈0.78 (Masutti et al., 2020).

Table 2. Colour parameters (L *, a *, b *), colour change (ΔE), moisture content and a_w of flavoured GF formulations (wafer with 100% honey, 50% honey, anise, vanilla and cinnamon coded GF-H100; GF-H50; GF-anise, GF-Vanilla and GF-cinnamon respectively); original GF wafer formulation (Or-GF) and wafer prepared with wheat flour (wheat).

	GF-H100	GF-H50	GF-Anise	GF-Vanilla	GF-Cinnamon	Or-GF	Wheat
Moisture	7.0 ± 0.4^{d}	5.4 ± 0.3^{cd}	4.1 ± 0.4^{bc}	3.9 ± 0.1^{bc}	3.6 ± 0.2^{b}	3.7 ± 1.7^{b}	2.0 ± 0.1^{a}
a_w	0.46 ± 0.01^{a}	0.49 ± 0.08^{a}	0.47 ± 0.02^{a}	0.45 ± 0.01^{a}	0.43 ± 0.01^{a}	0.45 ± 0.01^{a}	0.47 ± 0.01^{a}
Colour							
L*	35.4 ± 3.0^{a}	37.3 ± 5.6^{a}	39.8 ± 5.5^{a}	39.8 ± 8.9^{a}	50.0 ± 9.0^{b}	38.9 ± 5.4^{a}	50.9 ± 9.5^{b}
a*	9.6 ± 1.7^{a}	9.0 ± 2.2^{a}	12.9 ± 1.9^{b}	9.4 ± 1.0^{a}	13.5 ± 1.7^{b}	13.1 ± 2.0^{b}	10.4 ± 2.1^{a}
b*	6.5 ± 3.6^{a}	9.0 ± 6.6^{a}	22.7 ± 5.4^{b}	10.5 ± 8.8^{a}	28.5 ± 5.6^{b}	22.8 ± 5.0^{b}	27.98 ± 2.0^{b}
ΔE	361 ± 123^{b}	324 ± 214^{b}	104 ± 89^{a}	277 ± 203^{b}	52 ± 33^{a}	113 ± 99^{a}	41 ± 34^{a}
Texture							
σ (N/m^2)	225 ± 40^{b}	227 ± 117^{b}	251 ± 52^{b}	264 ± 128^{b}	106 ± 21^{a}	216 ± 26^{ab}	290 ± 77^{b}
ε	11.8 ± 7.2^{b}	5.6 ± 2.1^{a}	1.2 ± 0.1^{a}	4.6 ± 4.5^{a}	3.0 ± 0.9^{a}	1.4 ± 1.2^{a}	1.5 ± 0.7^{a}

*Results are expressed as mean ± standard deviation. Different letters within each column indicate significant differences among samples according to Fisher's test ($p \leq 0.05$).

Colour analysis. No significant differences were found in the L* parameter between the GF-Cinnamon and the wheat flour formulations ($p > 0.05$). Besides, these formulations were brighter than the Or-GF wafers and products with anise or honey addition ($p \leq 0.05$) (Table 3). Furthermore, the parameter ΔE showed that GF wafers with honey and vanilla presented the greatest difference with the wheat flour formulation ($p \leq 0.05$). Similar results were found by Conforti and Lupano (2004), who indicated that semi-sweet biscuits presented a darker appearance when honey was added to the formulation. Differences in colour can account for the non-enzymatic browning reactions that occur during the heating process of wafers. The colour differences in the honey formulations can be attributed to its high percentage of reducing carbohydrates. On the contrary, the other formulations contained sucrose, a non-reducing sugar, so the Maillard reactions were not favoured.

Moreover, it is well known that the rate of browning increases as the pH rises up to basic values (Ashoor and Zent, 1984). Thus, in order to explain the high luminosity of GF-Cinnamon formulations, the pH was measured. Results showed that this formulation presented the lowest pH value (5.67 ± 0.02) when it was compared with the Or-GF formulation (5.94 ± 0.01), GF-Anise (5.87 ± 0.01) and wheat flour (5.92 ± 0.01) formulations ($p \leq 0.05$). Therefore, it could be concluded that the GF-Cinnamon formulation presented higher L* values because the browning reactions were partially limited by the low pH.

Texture analysis. Table 2 displays the texture parameters of the wafers. The values of σ, which is related to the hardness of the product, were lower than the ones reported by Nasabi et al. (2021) in wafers prepared with the addition of non-wheat flour, but compare well with those reported by Tufan et al. (2020), who indicated that the hardness of wafers prepared with wheat flour and with chickpea replacement was between 1 and 3 N. Differences may account not only for the different formulations analysed, but also for the different dimensions of the products.

Furthermore, results showed that the Or-GF formulation and the GF-Cinnamon wafer presented the lowest values of σ ($p \leq 0.05$) (Table 3). Moreover, when sugar was fully replaced by honey (GF-H100), the strain (ϵ) significantly increased, which indicates a rubbery product ($p \leq 0.05$). This effect may be associated with the high moisture content of this formulation ($7.0 \pm 0.4\%$). In line with this result, Martínez-Navarrete et al. (2004) evaluated the plasticising effect of water in traditional wafers. These authors indicated that when the moisture levels increased, the product became more deformable and its crunchiness decreased. However, their results showed that the wafer texture was acceptable up to 11% of moisture content. This is slightly inconsistent with our present results, which indicate that GF wafers became rubbery when the moisture level was above 7.0%. The difference may be associated with the presence of wheat proteins in the traditional wafers analysed by Martínez-Navarrete et al. (2004).

Table 3. Sensory acceptability of the GF-Anise wafer, GF-cinnamon wafer and Or-GF wafer formulation.

	GF-Anise	GF-Cinnamon	Or-GF
Colour	6.87 ± 2.25^a	7.02 ± 2.12^a	7.38 ± 1.91^a
Taste	7.24 ± 2.25^a	6.74 ± 2.29^a	8.14 ± 1.61^b
Texture	7.92 ± 1.82^b	7.18 ± 2.09^a	8.02 ± 1.96^b
General Acceptability	7.54 ± 2.06^a	7.11 ± 2.02^a	8.31 ± 1.52^b

*Results are expressed as mean ± standard deviation. Different letters within each column indicate significant differences among samples according to Fisher's test ($p \leq 0.05$)

Selection of formulation by multivariate statistical tools. PCA was used to explore, in a more intuitive way, the data presented in Table 2 (Figure 1). The reports of the loadings of the variables, as well as the percentage of the explained data variation and the accumulated variation for the main components, can be found in the Supplementary Information.

As depicted in Figure 1 the two principal components, PC1 and PC2, explained 84.4% of the total variance. PC1 included most of the information concerning the b* value and the total colour difference (ΔE); in contrast, the fracture stress and a_w were mainly considered in PC2. This result indicated that the colour differences were responsible for the separation in PC1, while texture variations were associated with separations in PC2. This seems to indicate that the colour difference is a much better parameter to discriminate among these types of wafer formulations. Similar results were found by Diaz et al. (2019), who used PCA to study the effect of artichoke tuber flour as a wheat flour substitute for biscuit manufacturing. According to their findings, the primary component PC1 was better associated with the formulation of the biscuits and was mainly related to L* and b* colour parameters.

Moreover, the biplot displayed in Figure 1 indicated the presence of two main groups of wafer formulations that grouped together according to their similarities. The first group (G1), which presented negative values of PC1, included the following formulations: wheat flour, GF-Anise, Or-GF and GF-Cinnamon. The products of G1 showed high values of luminosity, were more reddish and were better characterised by high values of b*. On the other hand, the second group, G2 (formulations with vanilla and honey), showed high values of colour difference (ΔE), high moisture content and high values of fracture strain, which indicated a rubbery texture. These results agreed with data in Table 3. Moreover, the cluster analysis also indicated the presence of two major groups, G1 and G2, and confirmed that the most similar samples were the

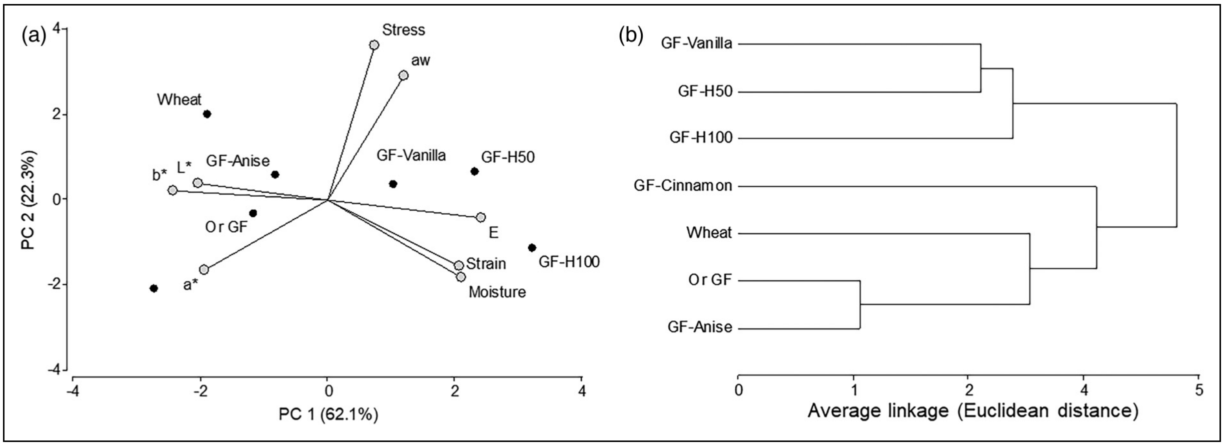

Figure 1. Results of PCA (a) and cluster analysis (b) of the seven different wafer formulations: wheat formulation (wheat), original GF (Or-GF), and GF with the addition of cinnamon (GF-Cinnamon), anise (GF-Anise) and honey at 50% or 100% of sugar replacement (GF-H50 and GF-H100 respectively).

original GF formulation and the anise wafer (Euclidian distance = 1.25).

According to these results, the addition of honey or vanilla to the wafer formulations is not recommended, because the products were very different to the control ones. On the other hand, the addition of cinnamon and anise did not significantly modify the physical characteristics of the product and would increase the antioxidant capacity of the formulations (see previous section). Thus, these formulations were selected for further analysis.

Sensory analysis. The acceptability of the selected wafer formulations with antioxidants was evaluated and compared to the Or-GF formulation (Table 3). Results showed no significant differences in the colour of the Or-GF, GF-Cinnamon or GF-Anise wafers (average values: 7.38 ± 1.91; 7.02 ± 2.12; 6.87 ± 2.25 respectively) ($p > 0.05$). This result indicates that, although significant differences were found in the brightness (L*) of the cinnamon wafers, this did not affect the acceptability scores assigned by the consumers.

On the other hand, the taste of the Or-GF formulation was better ranked than the GF-Cinnamon and GF-Anise (8.14 ± 1.61; 6.74 ± 2.26; 7.24 ± 2.25 respectively). A similar result was found in the general acceptability of these products (8.31 ± 1.52; 7.11 ± 2.02; 7.54 ± 2.06 for Or-GF, Cinnamon-GF and Anise-GF respectively). Moreover, excellent correlation was found between these two parameters ($r > 0.99$). Similar results were found by Olawoye and Gbadamosi (2020) in GF biscuits made from cardaba banana flour and starch blends. According to their results, the overall acceptability was better associated with the taste than with the texture or appearance of the products.

Moreover, although 65% of the consumers did not like the anise taste, while 16% expressed they did not like cinnamon, this did not affect the general acceptability of the GF-Anise or GF-Cinnamon formulations respectively (in both cases $p > 0.05$). Besides, it is important to underline that only 33% of the panellists were regular consumers of GF products; however, all the wafer formulations of the present work were GF and highly acceptable as the means were >6.7 on the hedonic scale. This indicates that the wafers would be well accepted not only by the celiac or GF market, but also by a wide range of consumers who seek healthy and good quality products.

Shelf-life analysis

Antioxidant content after in vitro digestion process. At the beginning of the storage period, the GF-Anise wafers presented a higher content of total phenlolic compounds than the GF-Cinnamon products (6.25 ± 0.05 GAE/g and 5.23 ± 0.64 GAE/g respectively) (Figure 2). These values were significantly higher than the ones reported by Canalis et al. (2020) in the bioaccessible fraction of biscuits with peach pulp addition (0.63 ± 0.05 mg GAE/g). The difference may be due to the different methods used to separate the insoluble and the bioaccessible fractions.

The ABTS method showed that the GF-Cinnamon wafers had the highest antioxidant capacity among all the formulations (43.5 ± 0.1 mg TE/g), while anise showed a more moderate antioxidant activity (18.8 ± 0.9 mg TE/g) ($p < 0.05$) (Figure 2). This result was slightly inconsistent with the data described in previous sections, which indicated that the antioxidant capacity of cinnamon was lower than that of anise according to ABTS. The differences could account for the effect of the heating treatment

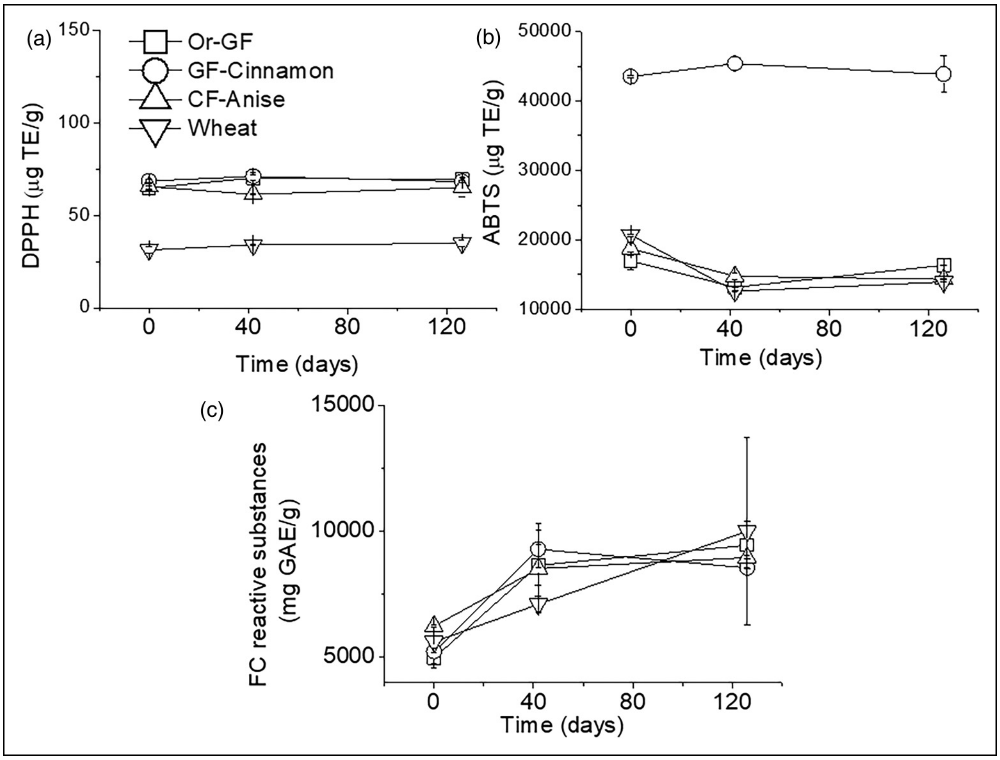

Figure 2. DPPH antioxidant capacity (a), ABTS antioxidant capacity (b) and the total phenolic compounds expressed as FC reactive substances (c) determined in the bioaccessible fractions of the different wafer formulations (Or-GF, GF-cinnamon, GF-anise and wheat) at different storage times. Results represent mean ± standard deviation (error bars).

during the preparation of the wafers and the effect of the digestion process. It is well known that high temperatures could induce the development of Maillard reaction products, and it has been indicated that melanoidins formed at the final stages could link phenolic compounds in their backbone (Pérez-Burillo et al., 2020). This could modify the original structure of the antioxidants and thus change their bioaccessibility. Moreover, there are some other factors that may also play an important role in the bioaccessibility of polyphenols in the digestive tract, such as pH transformations and interactions with other food components (Wojtunik-Kulesza et al., 2020).

The antioxidant activity of food products or beverages may change during the storage time. This effect has been mainly reported in fruits, vegetables and herbs, but there is little information about this in cereal-based foods (Naithani et al., 2006). This is a matter of great concern as it may affect the period in which the product could be consumed with its beneficial effects intact. Therefore, in the present work, the stability of the bioaccessible antioxidants in the wafers was evaluated at different storage times. Results can be found in Figure 2; according to DPPH assay no significant differences were found during the storage in the different formulations analysed ($p > 0.05$). However, the amount of total phenolic compounds and the antioxidant content determined by ABTS method showed significant differences during 4 months of conservation time ($p \leq 0.05$); nevertheless, their trends were opposite. ABTS technique showed that the content of antioxidants decreased over time, while the Folin technique showed that the content of bioaccessible phenolic compounds increased. Similar results were found by Ziegler et al. (2018) in rice grains stored for six months at different temperatures. These authors found an increase in the phenolic content as a function of storage time, but red rice grains showed a decrease in the antioxidant capacity against ABTS radical. The differences in the antioxidant content during storage may be caused by redox reactions between natural antioxidant phytochemicals or the development of Maillard reaction products, which may lead to different reactivity in the antioxidant methods used. On the whole, the highest antioxidant capacity was found in the GF-Cinnamon wafer formulation. Besides, its antioxidant capacity remained stable during the storage time.

Water absorption and physical modifications during storage time. Dry food systems can lose their desired textural properties during storage because of water absorption from the atmosphere into the product's matrix (Pestorić et al., 2017). Figure 3 depicts the increase in the water content and a_w of the wafers during six months of storage

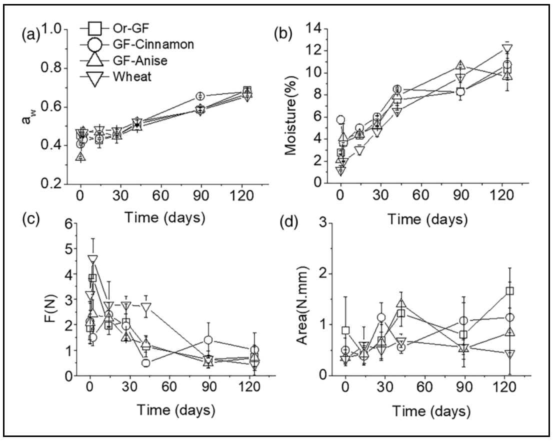

Figure 3. Water activity (a_W) (a), moisture content (b) and texture parameters (maximum force (c) and area (d)) determined in the different wafer formulations (Or-GF, GF-Cinnamon, GF-anise and wheat) at different storage times. Results represent mean ± standard deviation (error bars).

time at 25°C. No significant differences were found in the water absorption of the different wafer formulations during the length of the experiment ($p > 0.05$). The maximum a_w reached in the present work was low enough to avoid microbial growth, but the acceptability of the product may change. As depicted in Figure 3, during the storage time the fracture stress of all the products was significantly reduced, while the energy required to penetrate the sample (area, N.mm) significantly increased. These results indicate that the crunchiness of all the products was lost after four months of conservation time at 25°C because of water absorption ($p \leq 0.05$). Moreover, good negative correlations were found between the a_w and the fracture stress of the products ($r = 0.6$, $p \leq 0.05$). Cevoli et al. (2023) also found good correlations between the texture parameters of commercially available cream-filled wafers during storage and their a_W. According to these authors the association reflected the progressive plasticising effect of water, which caused a more deformable product with the increase in water mobility.

In the present work some differences were observed among the GF formulations and the wheat flour products. The fracture stress of the GF formulations (Or-GF, GF-Cinnamon and GF-Anise) decreased when the a_w value was 0.50 at day 45; while the texture of the wheat flour formulations remained stable until day 90, when the water activity was 0.65. Similar results were found by Martínez-Navarrete et al. (2004), who studied the effect of water activity on the texture of commercial wafers. Their results indicated that the critical water activity for the glass transition at 20°C was 0.59. Besides, they found that when the moisture level was higher than 11%, the glassy matrix turned fragile and the product became rubbery. This result is consistent with our findings in the wheat flour formulations, but suggests that the crunchiness of the GF products is more susceptible to water absorption.

CONCLUSIONS

It was possible to fully replace the fat generally used in wafer formulations by high oleic sunflower oil. According to the multivariate statistical analysis, GF formulations with the addition of cinnamon and anise were equivalent to the control ones prepared with wheat flour. Besides, these flavourings presented a high antioxidant capacity determined by DPPH, FRAP and ABTS.

The sensory analysis showed that the developed wafer formulations would be well accepted by a wide range of consumers, not only by celiac people. Besides, the highest antioxidant capacity was found in the GF-Cinnamon wafer formulation, which remained stable during the storage time, while its texture was acceptable until day 45.

Therefore, the present results provide relevant evidence that it is possible to develop good quality GF wafers with no addition of animal fat and with a high level of antioxidants. Nonetheless, these wafers should be combined with other products from various food groups, in order to diversify the GF diet and assure an adequate nutritional balance.

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SUPPLEMENTAL MATERIAL

Supplemental material for this article is available online.

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More references can be found in the Supplementary Information.