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Olive pomace oil improves the oxidative stability and nutritional value of oil-based cakes with anise essence, a traditional confectionery product in Spain

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ABSTRACT

Refined olive pomace oil (OPO) was studied as an alternative to sunflower oil (SO) in oil-based cakes with anise essence, a traditional confectionery baked product in Spain. Partial and total oil replacements in cakes obtained at an industrial level were evaluated. The influence of processing and storage on the oxidation extent and the contents of oil bioactive components was investigated. Results showed a low oxidative impact of the processing, which was significantly lower for OPO. Oxidation was however considerably high in SO at the end of the shelf-life (6 months), presenting peroxide values higher than 150 meq/kg oil, and very low in OPO, with peroxides close to the limit for fresh refined oils (5 meq/kg). Oil blends containing 25 or 50 wt% OPO exhibited intermediate results. Bioactive oil components practically remained unchanged in the processing, but significant losses of α-tocopherol were detected after 6-month storage, with 30–50% losses for SO and ~15% for OPO. The levels of squalene also remained high in OPO (90%) and no significant changes were found for sterols, triterpenic alcohols or triterpenic acids. A consumer panel test showed no differences between the fresh samples, but clear preferences for the new OPO-containing products were found during storage.

1. Introduction

Sunflower oil (SO) is a common vegetable oil frequently used in a number of confectionery foods such as biscuits, cupcakes, sponge cakes and others. The oxidative stability of conventional SO is limited owing to a relatively high content of linoleic acid, which can vary between 48% and 74% of total fatty acids (FAO-WHO, 1999).

Confectionery manufacturers have recently had to look for other vegetable oil alternatives to SO due to the shortage and price rise caused by the still ongoing armed conflict in Ukraine, one of the largest producing countries. In this regard, refined olive pomace oil (OPO) is examined in this research as one potential candidate that may enhance both the oxidative stability and nutritional value of confectionery foods. OPO is commercialised to the public as a blend of refined OPO with a small amount of virgin olive oil (VOO), which gives it characteristic flavours (IOC, 2022). However, refined OPO is a bland oil that can be used as an ingredient in the food industry.

OPO is a monounsaturated oil with high oxidative stability. Oleic acid in OPO can range between 55% and 85% of total fatty acids, whereas linoleic acid can oscillate between 2.5% and 21% (IOC, 2022). In a previous report, OPO has shown a high thermoxidative stability at frying conditions, which was greater compared to SO (Holgado, Ruiz-Méndez, Velasco, & Márquez-Ruiz, 2021).

Unlike conventional SO, OPO meets the fatty acid composition recommended in nutritional guidelines (EFSA, 2010; FAO-WHO, 2008) and it contains characteristic minor components of recognised health beneficial properties (Mateos, Sarria, & Bravo, 2020). Squalene, phytosterols, tocopherols, triterpenic alcohols such as erythrodiol and uvaol, triterpenic acids, mainly oleanolic acid, and aliphatic alcohols are bioactive components in OPO. A few of these are present in much lower amounts in SO, like squalene, or they are not found at all, as they are unique to OPO and other olive oils, such as triterpenic compounds and aliphatic alcohols (Márquez-Ruiz & Holgado, 2018). Beneficial health effects of long-term OPO consumption have been reported recently. Hypolipidemic effects, reduction of total and LDL cholesterol, and reduction of waist circumference have been found in healthy subjects

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and individuals at cardiovascular risk, contributing to cardiovascular and cardiometabolic disease prevention. These effects were not found when SO or high-oleic SO were used (González-Rámila et al., 2022a, 2022b, 2022c).

Whilst there are certain scientific precedents of the thermoxidative stability of OPO in frying of foods (Holgado et al., 2021; Ruiz-Méndez, Márquez-Ruiz, Holgado, & Velasco, 2021), little is known about oxidative changes of refined OPO at baking conditions. Caponio, Giarnetti, Paradiso, Summo, and Gomes (2013) investigated different oils in a traditional Italian savoury product rich in fat, named *Taralli*, which is made with flour, water, oil, salt and spices. OPO, which was used headed with VOO, showed a high oxidative stability that was comparable to olive oil and refined palm oil. In a recent report, refined OPO was compared to SO in cupcakes (Velasco, García-González, Zamora, Hidalgo, & Ruiz-Méndez, 2023). Results showed that even when the oxidation extent of the fat material was low during the processing and storage, the refined OPO reduced considerably the levels of lipid oxidation compared to SO.

Oil-based cakes with anise essence have been selected in this study because of their elevated content of SO (22–23 g/100 g). They are a confectionery baked product traditionally made in Spain with wheat flour, water, oil, sugar, sesame seeds, aniseed, yeast, salt and anise essence. A dough made with these ingredients is baked at elevated temperature to obtain a thin, round-shaped cake with a crunchy texture. The most known one is the Sevillian oil-based cake, made with extra virgin olive oil (EVOO) and manufactured since 1910 by a Sevillian producer. Since then this has been a benchmark in the confectionery sector. Different variants of this cake, like the one of the present work containing SO instead, can be found throughout Spain, either home-made or factory-made by small and large manufacturers. Refined OPO is a bland oil that could be a healthier alternative to SO and compared to EVOO is relatively inexpensive and free of characteristic flavours, which is essential for the regular consumers of cakes made with refined SO.

The thin thickness of oil-based cakes may favour the air exposure of the oil during baking. In addition, due to the low water content, the dough could reach relatively high temperatures that easily may induce oxidative degradation and compromise the oil stability and also the nutritional value, reduced for both the formation of oxidised lipids with potential toxicity (Grootveld, Percival, Leenders, & Wilson, 2020) and possible losses of oil bioactive components.

As to the bioactive components of OPO, their thermoxidative stability at frying conditions has been recently reported (Ruiz-Méndez et al., 2021). It was found that tocopherols and squalene degraded fast at elevated temperature, whereas sterols, oleanolic acid, erythrodiol and especially aliphatic alcohols exhibited a great resistance to thermoxidative degradation. The resistance to thermoxidative degradation of these components at baking conditions has been studied in cupcakes in a previous report (Velasco et al., 2023). Results exhibited a high stability of OPO bioactive components during the processing and also the storage of cupcakes, which both had a low impact on the oxidative degradation of the fat material. Slight losses of squalene (8% by weight) and α-tocopherol (13% by weight) were found, respectively, in OPO after the processing and storage of the cupcakes until the end of the shelf life. No significant losses of sterols, triterpenic acids or triterpenic alcohols were found in the study.

In this research we evaluate the influence of partial and total replacement of SO with refined OPO on the oxidative stability and nutritional value of oil-based cakes produced at an industrial level. As outlined above, the characteristics of this product may favour oxidative changes and compromise the oil stability and so the food quality during the shelf life. It was hypothesised that OPO could improve the quality and nutritional value of sunflower oil-based cakes owing to its high resistance to oxidative degradation and its valuable bioactive components. The influence of both the processing and storage under realistic conditions on the oxidation extent and the contents of oil bioactive components were investigated. In addition, the acceptability of the new products was also evaluated by consumer panel tests to finally prove the realistic potential applications of refined OPO in the confectionery food sector.

2. Materials and methods

2.1. Oils and cakes

Three refined SOs and three refined OPOs were used in this study. The oils were purchased from a local supplier (COREYSA, S.A.) except one of the SOs (SO3), which was the one in use by the manufacturer of cakes at that moment. From blends of SO and OPO, three new oil samples containing 25% by weight OPO (OPO25) and other three containing 50% by weight OPO (OPO50) were obtained. Twelve different cake samples were produced at an industrial scale by a local manufacturer of these products (Table S1) (San Martín de Porres, S.L.). Three different batches produced under normal operation conditions from SO3 were taken as control samples. These were not experimental samples, but real cakes taken from the production chain of the manufacturer. The control cakes belonged to batches that were placed on the market. Apart from the oil, the new cakes were produced exactly the same. Nine different samples containing OPO, OPO25 or OPO50 were manufactured, each one with each oil (Table S1). The ingredients were those employed in the manufacture of these products, i.e. wheat flour, water, oil, sugar, sesame seeds, aniseed, yeast, salt and anise essence. The proportions of major components in the dough were 49.8 g/100 g flour, 18.6 g/100 g oil, 15.9 g/100 g water and 13.28 g/100 g sugar. The amount of dough prepared in each batch was 130 kg. Kneading was performed at room temperature for 15 min. Then the dough was processed in a continuous production line comprising a tunnel oven using temperatures from 389 °C to 500 °C above the conveyer belt and from 180 °C to 380 °C below. The dwell time in the oven was 6–8 min. The cakes were packed in usual packages of 6 units with a net weight of 180 g. Each unit was wrapped in paraffin paper and then the 6 units were piled in a PET tray and packed in polypropylene packaging.

2.2. Oil characterisation

The composition of fatty acids, sterols, triterpenic alcohols, and fatty alcohols were determined by applying the EEC regulation (EEC, 1991).

The oils were transmethylated with KOH in methanol at room temperature to obtain the fatty acid methyl esters (FAME). Then the composition of fatty acids was determined by gas chromatography (GC) analysis of the FAME. For details the reader is referred to Supplementary Material (Method S1).

Sterols and triterpenic alcohols, and fatty alcohols were analysed from the unsaponifiable fraction of 250 mg oil using 5-α-cholestan-3-ol and n-heneicosanol as internal standards. They were isolated by thin layer chromatography (TLC), derivatised to trimethylsilyl ether derivatives, and analysed by GC (Method S2).

Squalene was analysed applying a contrasted method which is based upon the International Olive Council method for the content of waxes, fatty acid methyl esters, and fatty acid ethyl esters by GC (IOC, 2022). Squalene was isolated by adsorption chromatography using a column packed with silica and analysed by GC (Method S3).

Triterpenic acids were analysed according to the method by Pérez-Camino and Cert (1999). They were separated by solid-phase extraction (SPE) using an SPE-NH_2 cartridge, derivatised to trimethylsilyl ether derivatives, and analysed by GC (Method S4).

Tocopherols were analysed by HPLC with fluorescence detection according to ISO 9936:2016 method (ISO, 2016). An Agilent Technologies 1260 HPLC chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump, an autosampler, a thermostatted column compartment, a silica HPLC column (LiChrospher® Si 60, 250 mm × 4 mm, 5-μm particle size) (Merck, Darmstadt, Germany) and a fluorescence detector was used. Heptane:2-propanol (99:1, v/v) at

1 mL min^{-1} was the mobile phase. The separation was performed at 25 °C. The excitation and emission wavelengths were set at 290 and 330 nm, respectively. External calibration was applied for quantification.

Acidity and peroxide value (PV) were analysed by ISO 660:2020 (ISO, 2020) and ISO 3960:2017 (ISO, 2017) methods, respectively.

The oxidative stability index (OSI) was determined from 2.5 g oil in the Rancimat test at 100 °C and 20 L/h air according to AOCS Cd 12b-92 (AOCS, 2022).

Triglyceride linoleate hydroperoxides or hydroperoxydienes were analysed by HPLC-UV following the method of Velasco, Morales, Ruiz-Méndez & Márquez-Ruiz (2018). A solution of 50 mg/mL of oil in n-heptane was directly analysed in an AT 1260 Infinity HPLC chromatograph (Agilent Technologies Inc., CA, USA) equipped with a silica HPLC column (LiChrospher® Si 60, 250 mm × 4 mm i.d., 5 μm particle size) (Merck, Darmstadt, Germany) and a diode array detector (DAD). Isocratic elution with n-heptane:diethyl ether (82:18, v/v) was applied. Hydroperoxydienes were detected at 234 nm and quantified using a response factor determined elsewhere (Velasco, Morales-Barroso, Ruiz-Méndez, & Márquez-Ruiz, 2018).

2.3. Cake characterisation

From cake samples ground in a mortar and pestle, the moisture content was measured in an Ohaus MB45 analyser using a halogen heating source (Ohaus Corporation, Parsippany, NJ, USA).

The oil content was obtained from samples ground in a mortar and pestle by direct Söxhlet extraction with hexane for 6 h according to method ISO 734:2015 (ISO, 2015).

Colourimetric measurements on cakes were done using a PCE-CSM8 spectrophotometer (PCE Instruments, Alicante, Spain) that provides the CIE L* (lightness), a* (redness) and b* (yellowness) parameters. Measurements were performed on three points of a cake and 9 cakes were taken for each product. The colour index (CI) was calculated applying the formula CI = L*(b*-a*)/100 according to Castellano, García, Morilla, Perdiguero, and Gutiérrez (1993).

The OSI was determined using 5 g ground cake in the Rancimat test at 100 °C and 20 L/h air according to AOCS Cd 12b-92 (AOCS, 2022).

2.4. Oil extraction

Fresh dough samples were frozen at −32 °C, freeze-dried in a Lyo-Quest lyophilizer (Telstar S.A., Madrid, Spain), and ground in a mortar and pestle. The water content removed by freeze-dried was 30.5 ± 0.9 g/100 g. The oil extracts were obtained from 30 g of ground samples by applying a solid-liquid extraction with 100 mL n-hexane at room temperature. Stirring for 10 min under nitrogen and darkness conditions was applied. After filtration onto filter paper (75 g m^{-2}) the sample was washed twice with 50 mL n-hexane each. The solvent was removed in a Büchi R-210 rotary evaporator (Büchi Ibérica, Barcelona, Spain) at 35 °C. Trace hexane was finally removed with a stream of nitrogen. The oil extracts from cakes were obtained from 20 g ground samples following the same procedure described for the dough, except that no treatment was applied to remove the moisture.

2.5. Oxidative deterioration in the processing

The dough making and baking processes were examined by comparing the dough extracts to the fresh oils and cake extracts, respectively. The PV was used for the former, while the levels of polar compounds by HPSEC analysis (Dobarganes, Velasco and Dieffenbacher, 2000) (Method S5), the PV and the contents of hydroperoxydienes, as described above, were used to evaluate the baking process. The concentrations of α-tocopherol, squalene, sterols, triterpenic alcohols, and triterpenic acids were analysed to evaluate possible losses in the baking.

2.6. Storage under controlled conditions

Selected cake samples, packed in original packaging of 6 units, were stored in a room under darkness conditions and analysed periodically during the shelf-life, which was 180 days according to the manufacturer, and after 3 additional months. The room temperature was 23 ± 2 °C, with minimum and maximum temperatures of 16.5 °C and 26.5 °C, respectively. The samples studied were the three control samples containing SO3, OPO25-1, OPO50-1 and OPO-1.

2.7. Oxidative degradation in the storage

Primary oxidation was evaluated by applying the PV and also the analysis of hydroperoxides by HPLC described above. The content of hexanal was determined as a marker of secondary lipid oxidation. It was analysed by SPME-GC-MS using a Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) SPME fibre (Supelco, Bellefonte, PA, USA) (Method S6).

The cake samples were also assessed by sensory analysis. The analyses were carried out by three expert panellists of the official virgin olive oil panel of the Instituto de la Grasa (CSIC). In an open session, the panellists evaluated rancidity on cakes taken from a given package. First, they smelled a cake and then tested it to assess the aroma and flavour, respectively. The intensity of perception was measured on a structured 9-point scale. The intensity represented by the score was 0-not perceptible, 2-slightly perceptible, 4-perceptible, 6-considerably perceptible, 8-strongly perceptible and 9-very strongly perceptible. Only samples with intensities of 4 or higher showed rancid flavour. When the score was lower than 4, rancidity was perceived in the aroma, i.e. via orthonasal, but not by via retronasal. SO oxidised at 60 °C for 1 week and its dilutions with fresh oil were used as references.

The concentrations of oil bioactive components were also analysed to evaluate possible losses over storage.

2.8. Acceptability of the fresh cakes by consumer panel tests

The fresh cake samples coded with reference 1, i.e. SO3-1, OPO25-1, OPO50-1 and OPO-1, were assessed by a consumer panel to examine whether the type of oil affects the acceptance and preferences of consumers. The panel was made up of 60 consumers (31 women and 29 men) and the tests were performed by a specialised company (AINIA Consumer, Paterna, Spain). The attributes assessed were global appraisal, appearance, colour, odour, flavour, taste intensity, sweet taste, texture and crunchy texture. The consumers were also asked to indicate whether they perceived rancid odour or flavour of seed oils or nuts and to rank the samples in order of preference. For more details see Method S7.

2.9. Acceptability of cakes during storage by consumer panel tests

The samples stored for 3 and 5 months were also evaluated internally by consumer panels of 31 and 63 individuals, respectively. The first panel consisted of 14 men and 16 women, aged 23–39 (16%), 40–49 (26%) and 50–63 years old (58%), whereas the second one comprised 30 men and 33 women, aged 20–39 (24%), 40–49 (29%) and 50–66 years old (47%). They were given the same questionnaire as that used for the fresh samples and proceeded the same.

2.10. Statistical analyses

The fresh oils and dough extracts were analysed in triplicate. Unless indicated, the cakes were also analysed in triplicate. Thus, three cakes from a same package were analysed only once. Results were expressed as the mean values ± standard deviations. One-factor ANOVA was applied in this study. Levene's test based on the mean value was used to verify that variances were equal across groups. Duncan's test or Kruskal-Wallis

non-parametric test was applied for multiple mean comparisons. Student's *t*-test was applied when two samples were compared. A paired-*t* test was applied to evaluate the effect of kneading on the PV. Friedman's test was applied to determine significance in the differences obtained in the consumer preference tests. When significant, a post-hoc Wilcoxon signed ranked test was applied for multiple pair-wise comparisons. Significance was defined at $p < 0.05$. The analyses were performed using 29.0 IBM SPSS Statistics program (IBM Corp., Chicago, IL, USA).

3. Results and discussion

3.1. Oil characterisation

The fatty acid compositions of the oils were characteristic of high-linoleic SOs (FAO-WHO, 1999) and oils derived from olives (IOC, 2022), respectively (Table S2). Those of the oil blends were expected from the oil proportions used (Table S3). The bioactive components analysed in the SOs, i.e. sterols, squalene and tocopherols, were within normal ranges (Table S4). Those determined in the OPOs were sterols, triterpenic alcohols, squalene, triterpenic acids, fatty alcohols and tocopherol, and their levels were also within reported ranges (Holgado et al., 2021; Márquez-Ruiz & Holgado, 2018). As expected from refined oils, low amounts of triterpenic acids were found (60–118 mg/kg) and only oleanolic and ursolic acids were detected.

The acidity and PV indicated that the oils were fresh and of high quality, with results of 0.07–0.11% for the former and PVs lower than the limits established for fresh refined seed oils (<10 meq/kg) or refined OPOs (<5 meq/kg) (Table S5).

The OSI values for the SOs (10.3–12.1 h at 100 °C) and OPOs (20.3–38.8 h at 100 °C) (Table S5) were within normal ranges (Holgado et al., 2021). Expectedly, the oil blends showed intermediate values (Table S6).

3.2. Cake characterisation

The cake samples displayed low moisture contents (1.8–3.1 g/100 g) and the oil amount was 22.3 ± 0.5 g/100 g (Table S7). Significant differences were not found for the moisture content between samples prepared with OPO25, OPO50 and OPO, but their moisture was slightly lower (1.9 ± 0.4 g/100 g) compared to the control samples (2.5 ± 0.5 g/100 g). No significant differences were found either for the fat content ($p = 0.081$) or colour index ($p = 0.189$) (Table S7). Therefore, the samples presented similar characteristics of moisture, fat content and colour.

The OSI values determined directly in the cakes were comparable to those of the oils, although higher for the OPO samples (Table S7). Significant differences were found between samples containing different types of oil, with mean values of 9.6, 11.7, 16.6 y 36.3 h for SO, OPO25, OPO50 and OPO, respectively.

3.3. Oxidative degradation and relative losses of bioactive components in the processing

No significant lipid oxidation was found in the dough making step. The PV of the oils did not increase significantly in a consistent way (Fig. S1). In fact, a paired-*t* test did not show significant differences between the oil and dough ($p = 0.180$). This is not the case in products obtained from a batter such as muffins and sponge cakes, showing clear enzymatic oxidation during the beating (Krause, Keller, et al., 2022; Maire, Rega, Cuvelier, Soto, & Giampaoli, 2013). The lipids in a batter are emulsified, exhibiting a large contact surface that favours reactions, while in the cake dough of this study the oil forms a continuous phase of much lower specific surface. In addition, inclusion of air as bubbles during beating also contributes to lipid oxidation in sponge cakes (Krause, Asamoah, Moulin, Bonazzi, & Rega, 2022).

The kneading step did not cause either significant losses of oil bioactive components (results not shown). During the kneading step of breadmaking, a few authors have observed however slightly losses of Vitamin E activity, provided by the very low contents of tocopherols in the wheat flour, and these were attributed to direct oxygenation (Leenhardt et al., 2006). In contrast, the dough of the present study contained a great amount of oil protected naturally with elevated levels of α-tocopherol. If tocopherol losses occurred, they were below the analytical error (<5%).

The contents of polar compounds, analysed in the SO and OPO samples, were used to evaluate the impact of the baking process. Results did not show in any case significant differences between the cake and dough extracts (Table 1). Formation of polymers was not observed and increased oxidised triglyceride levels were significant only for the SO3-2 sample, suggesting that oxidation was low and that the analysis of polar compounds was not sensitive enough to detect significant changes.

Table 1
Influence of the baking process on the levels of polar compounds (g/100 g oil extract) in cakes prepared with sunflower oil (SO) or olive pomace oil (OPO).

Sample		TGD	oxTGM	DG	MG	FFA	TPC
SO3-1	Dough	0.54 ± 0.02a	2.13 ± 0.69a	1.24 ± 0.06a	0.25 ± 0.00a	0.68 ± 0.01a	4.84 ± 0.68a
	Cake	0.62 ± 0.08a	2.04 ± 0.09a	1.20 ± 0.03a	0.26 ± 0.01b	0.67 ± 0.08a	4.79 ± 0.13a
SO3-2	Dough	0.52 ± 0.07a	1.28 ± 0.20a	1.12 ± 0.09a	0.27 ± 0.00a	0.69 ± 0.08a	3.87 ± 0.24a
	Cake	0.60 ± 0.02a	1.64 ± 0.17b	1.11 ± 0.04a	0.27 ± 0.00a	0.64 ± 0.04a	4.27 ± 0.12a
SO3-3	Dough	0.53 ± 0.03a	1.34 ± 0.07a	1.13 ± 0.04a	0.29 ± 0.00a	0.76 ± 0.08a	4.06 ± 0.11a
	Cake	0.60 ± 0.05b	1.53 ± 0.11a	1.22 ± 0.06a	0.29 ± 0.01a	0.74 ± 0.06a	4.38 ± 0.22a
OPO-1	Dough	1.12 ± 0.14a	1.68 ± 0.26a	5.91 ± 0.34a	0.68 ± 0.02a	0.87 ± 0.05a	10.26 ± 0.59a
	Cake	1.07 ± 0.05a	1.59 ± 0.12a	6.52 ± 0.56a	0.70 ± 0.02a	0.87 ± 0.13a	10.75 ± 0.76a
OPO-2	Dough	1.30 ± 0.14a	2.29 ± 0.39a	8.12 ± 0.17b	0.68 ± 0.05a	0.95 ± 0.05a	13.35 ± 0.09a
	Cake	1.24 ± 0.04a	2.12 ± 0.45a	7.61 ± 0.02a	0.65 ± 0.05a	0.94 ± 0.09a	12.57 ± 0.57a
OPO-3	Dough	1.11 ± 0.14a	1.94 ± 0.59a	7.59 ± 0.89a	0.73 ± 0.05a	0.73 ± 0.03a	12.11 ± 1.33a
	Cake	1.25 ± 0.08a	2.24 ± 0.28a	7.91 ± 0.49a	0.73 ± 0.01a	0.85 ± 0.07b	12.98 ± 0.53a

TGD, triglyceride dimers; oxTGM, oxidised triglyceride monomers; DG, diglycerides; MG, monoglycerides; FFA, free fatty acids and other polar minor components; TPC, total polar compounds. Results represent the mean value followed by the standard deviation of 3 analytical determinations in an only oil extract (Dough) or in oil extracts of 3 independent samples (Cake). Different letters for a given group of compounds indicate significant differences between the dough and cake according to Student's *t*-test ($p < 0.05$).

Hydrolysis of the oil was not detected either, as partial glycerides and free fatty acids remained unchanged. Similar results have been found in biscuits, in which oxidation during kneading and baking was so low that the analysis of polar compounds appeared not to be sensitive enough (Caponio, Summo, Pasqualone, & Bilancia, 2008).

The results obtained for primary oxidation products did exhibit oxidative degradation in the baking process (Fig. 1). The PV increased significantly and in a much greater extent in the samples containing SO, neat or blended with OPO. The PV in the OPO samples was remarkably low, with results below 3.5 meq/kg, that is below the limit for fresh refined OPOs (5 meq/kg) (Fig. 1A). Alternatively, the contents of triglycerides bearing linoleate hydroperoxides were also evaluated by applying a direct HPLC method developed in our lab (Velasco et al., 2018). This was used to verify that the PV was not affected by possible analytical interferences due to the partial coextraction of food components of either reducing or oxidising nature (Velasco et al., 2023). In this regard, the analysis of hydroperoxydienes also showed similar results to those found for the PV. Their levels rose considerably in all cases in the baking process, except in the OPO samples, whose increased levels were close to the initial values (Fig. 1B).

As to the bioactive components, slight significant losses in the baking process were only observed for tocopherol, and not in a systematic way because different results, significant and not significant, were found for samples prepared with the same type of oil. When significant, these were higher for the SO and OPO25 samples, with losses ≤12% by weight, whereas those in OPO50 and OPO were ≤8% by weight (Table 2). Changes in the squalene content were below the analytical error (5%) and therefore they were not significant (Table 2). Significant changes were not observed either in the content of sterols (Table S8), triterpenic alcohols or triterpenic acids in the samples analysed (Table S9).

The results presented above clearly show that the baking process had

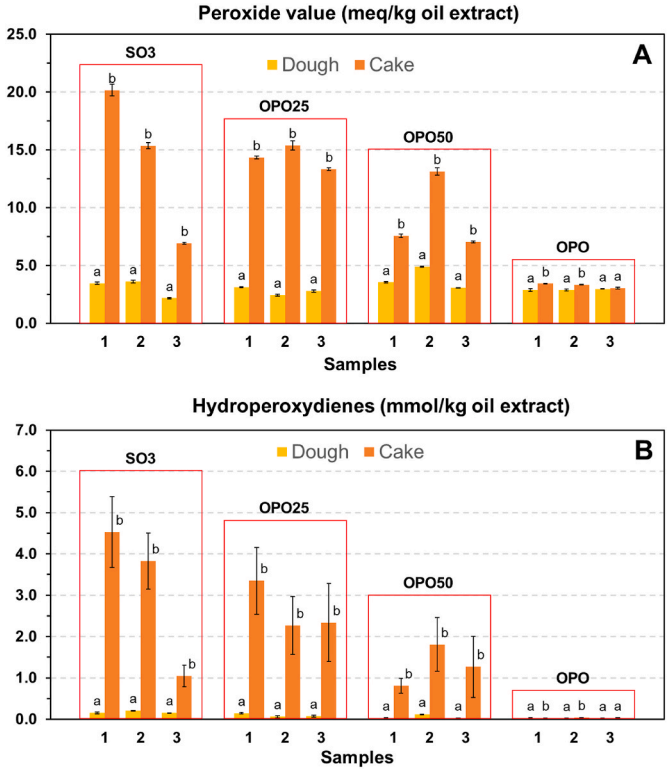

Fig. 1. Influence of the baking process on the peroxide value (**A**) and concentrations of hydroperoxydienes (**B**). Results represent the mean and standard deviation of 3 analytical determinations in an only oil extract (Dough) or in oil extracts of 3 independent samples (Cake). Different letters indicate significant differences between the dough and cake according to Student's *t*-test ($p < 0.05$).

Table 2
Influence of the baking process on the levels of α-tocopherol and squalene.

Sample	α-Tocopherol (mg/kg oil extract)		Squalene (mg/kg oil extract)	
	Dough	Cake	Dough	Cake
SO3-1	734 ± 11b	663 ± 8a	174 ± 6a	173 ± 4a
SO3-2	727 ± 5b	640 ± 12a	190 ± 13a	183 ± 8a
SO3-3	655 ± 6a	665 ± 18a	216 ± 19a	266 ± 14b
OPO25–1	613 ± 2b	547 ± 7a	436 ± 18a	460 ± 15a
OPO25–2	588 ± 3b	520 ± 17a	401 ± 10a	443 ± 45a
OPO25–3	599 ± 3b	551 ± 25a	473 ± 3a	497 ± 13b
OPO50–1	468 ± 5b	431 ± 15a	748 ± 56a	840 ± 74a
OPO50–2	471 ± 41a	445 ± 15a	563 ± 12a	632 ± 17b
OPO50–3	498 ± 4b	470 ± 16a	827 ± 6a	874 ± 22b
OPO-1	377 ± 4b	352 ± 12a	1523 ± 13b	1464 ± 31a
OPO-2	334 ± 2a	344 ± 20a	1149 ± 37a	1058 ± 36a
OPO-3	362 ± 6b	347 ± 4a	1673 ± 13a	1694 ± 58a

Results represent the mean value followed by the standard deviation of 3 analytical determinations in an only oil extract (Dough) or in oil extracts of 3 independent samples (Cake). Different letters in a row for a given bioactive compound indicate significant differences between the dough and cake according to Student's *t*-test ($p < 0.05$).

a small or moderate impact on the lipid composition of oil-based cakes, giving rise to relatively small or moderate quantities of primary oxidation products and slight losses of α-tocopherol. Even though the temperatures applied in certain zones of the tunnel oven were as high as 500 °C, the thickness of the dough was rather thin, and the loss of humidity was considerable, the oxidative degradation was only moderate. It can be deduced therefore that the baking time was so short (6–8 min) that the oil did not change substantially. Yet, the levels of hydroperoxides were definitely much lower in the OPO samples, with values within the range of fresh oils, whereas samples containing SO, neat or blended with OPO, showed PV higher than the limit for fresh refined seed oils (10 meq/kg). It is known that the levels of oxidised lipids contribute to reducing the oil oxidative stability, as lipid oxidation is an autocatalytic process (Frankel, 2005), and may have an impact on quality during storage.

3.4. Consumer acceptability of the new products

The results obtained for each of the attributes assessed did not show significant differences between the new cakes containing OPO, neat or blended with SO, and the control containing SO (Table S10). The preference test did not show significant differences either (Fig. S2). Therefore, none of the samples were preferred to the others. In conclusion, the type of oil did not affect the organoleptic properties of the cake or the consumers did not distinguish between products elaborated with different oils.

According to a database of sensory acceptance of over 4000 tested foods (Method S6), the cakes were well assessed, receiving elevated scores of well assessed (6.1–6.7) and very well assessed (>6.7) foods.

3.5. Resistance to oxidative degradation and losses of bioactive components during the shelf-life

3.5.1. Oxidative degradation

Relatively high lipid oxidation was found in the samples containing SO, either neat or blended with OPO, at the end of the shelf-life (6 months). The PV in the control samples was higher than 150 meq/kg oil, whereas that in the OPO sample was still close (5.9 meq/kg) to the limit for fresh refined oils (5 meq/kg) (Fig. 2A). The samples containing the oil blends presented intermediate PVs, being higher for the sample with lower OPO content (OPO25). The hydroperoxide results by HPLC were

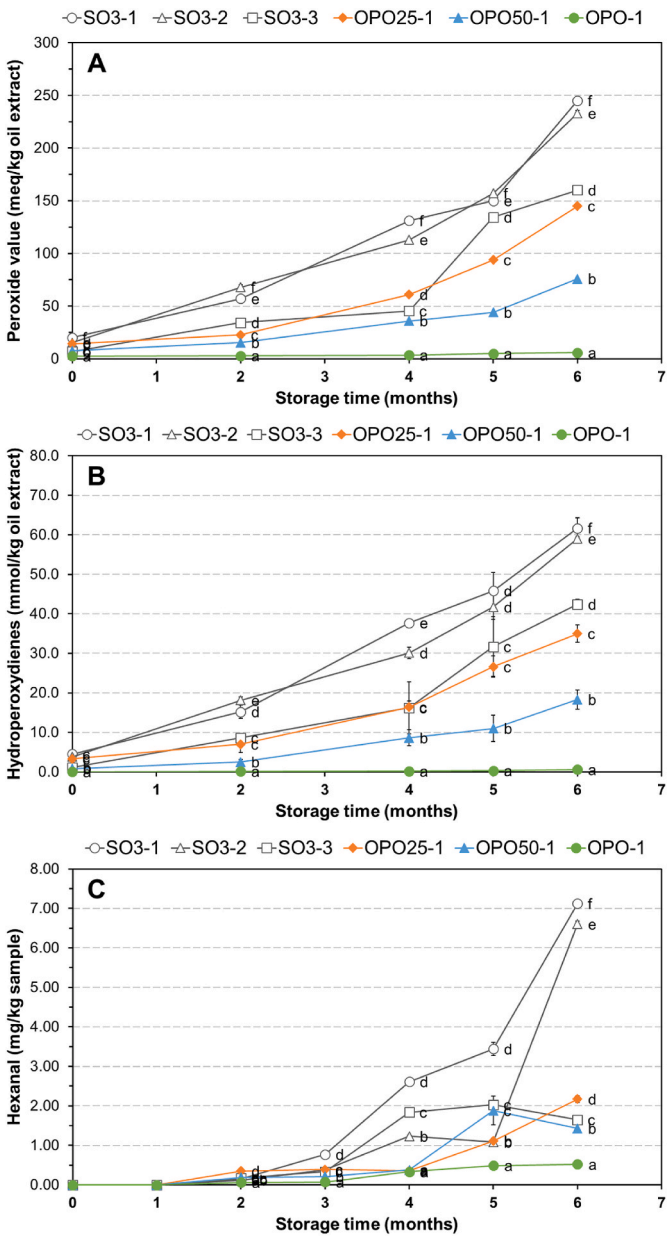

Fig. 2. Changes in peroxide value (**A**), hydroperoxydienes (**B**) and hexanal (**C**) during storage. Results represent the mean and standard deviation of 3 independent cakes. Different letters indicate differences between samples according to Duncan's test ($p < 0.05$).

similar to those for the PV, with very high values after 6-month storage for the SO samples, followed by OPO25 and OPO50, and extremely low for OPO (Fig. 2B). As expected from the high primary oxidation reached, very substantial increases in hexanal, a hydroperoxide degradation product contributing to rancid off-flavours, were observed in all cases except in the OPO sample (Fig. 2C). The hexanal levels increased considerably with the degree of oil unsaturation, with high levels for the SO samples and very low for the OPO sample.

All the samples displayed very high PVs after 9-month storage, with the exception of OPO-1, which was relatively low (21.6 meq/kg). Similar results were found for hydroperoxydienes, proving that this is a good analytical alternative to the PV determination (Fig. S3).

The high resistance to oxidative degradation of refined OPO over processing and storage of oil-based cakes is consistent with the works by Caponio et al. (2013) and Velasco et al. (2023). The former studied different vegetable oils in a traditional Italian savoury food rich in fat, named *Taralli*. The PV found after 5-month storage in *Taralli* samples containing OPO, i.e. refined OPO headed with virgin olive oil, was not significantly different (21.8 meq/kg oil) from those obtained in samples made with olive oil (19.5 meq/kg oil) or refined palm oil (19.9 meq/kg fat), i.e. two fats of elevated oxidative stability. In a previous report, it was found that the levels of oxidised lipids were considerably reduced in cupcakes with the substitution of SO with refined OPO. The concentrations of linoleate hydroperoxides determined by HPLC in the cupcakes at the end of the shelf life were decreased from 10.90 (±0.7) mmol/kg fat in the control containing SO to 0.25 (±0.7) mmol/kg fat in the sample containing OPO (Velasco et al., 2023).

3.5.2. Sensory evaluation

Sensory analysis was performed in the 12 cake samples. The results were coherent with those obtained for the different oxidative parameters studied. Oxidative rancidity was perceived after 4-month storage in the SO and OPO25 samples (Fig. S4). At this time, slight rancidity was detected via orthonasal, but not retronasal, probably masked by the intense anise flavour. After 6-month storage, oxidative rancidity was more intense in the aroma of both samples and only the three SO samples also exhibited rancidity via retronasal. Similarly, slight rancidity was perceived via orthonasal, but not retronasal, in the OPO50 samples after 5-month storage. Regarding the OPO samples, OPO-1 showed no evidence of rancidity throughout the study, even after 9-month storage, while OPO-2 and OPO-3 presented a similar behaviour to the OPO50 samples, with slight rancidity in the aroma after 5-month storage. It is noteworthy that OPO-1 was prepared with the oil that presented the highest oxidative stability in the Rancimat test (Table S5). After 9-month storage, the panellists unanimously reported clear unpleasant off-flavour in the SO and OPO25 samples, whereas the OPO50 and OPO samples showed satisfactory flavours, although with clear losses of anise flavour intensity.

3.5.3. Losses of oil bioactive components

The cake samples presented substantial losses of tocopherol at the end of the shelf-life (6 months) (Fig. 3A). The highest losses were found in SO3-1 and SO3-2 (~50% by weight), followed by SO3-3 and OPO25-1 (~30% by weight). Tocopherol losses of only 15% by weight were found for the OPO50-1 and OPO-1 samples. At the end of the test, that is 3 months after the shelf-life, the SO3-1 sample had completely lost tocopherol, due to the advanced state of oxidation. However, 80% by weight of this antioxidant remained in OPO-1 at the same period, while OPO50-1 presented losses of 45%. The results obtained for squalene also showed significant losses at the end of the shelf-life (Fig. 3B). The lowest losses were found for OPO-1, which lost 8% by weight squalene, whereas losses that ranged between 15% and 20% by weight were found for the rest of the samples. No significant changes of sterols (Table S11), triterpenic alcohols or triterpenic acids (Table S12) were observed.

3.6. Consumer acceptability of the new products during the shelf-life

Apart from the flavour score, which was worse assessed for the SO sample, no significant differences were found in the attributes evaluated between the 4 samples stored for 3 months (Table S13). Consumers showed clear preferences for the new samples containing OPO (Fig. S5A). Only 6% of the panellists chose the SO3-1 sample in the 1st position, whereas almost half of them selected it as the last preference. The differences with respect to the control were more pronounced in the samples stored for 5 months. The results showed a higher overall appraisal for the three OPO-containing cakes (Table S14). Ratings for colour, odour, flavour, and texture were also higher compared to the control. No clear differences were observed between the three cakes containing OPO. Once again, consumers showed clear preferences for them (Fig. S5B). As shown throughout this study, the worst assessments of the control sample were related to oxidative degradation. In fact, the frequency of the perception of rancid odour or flavour of seed oils or

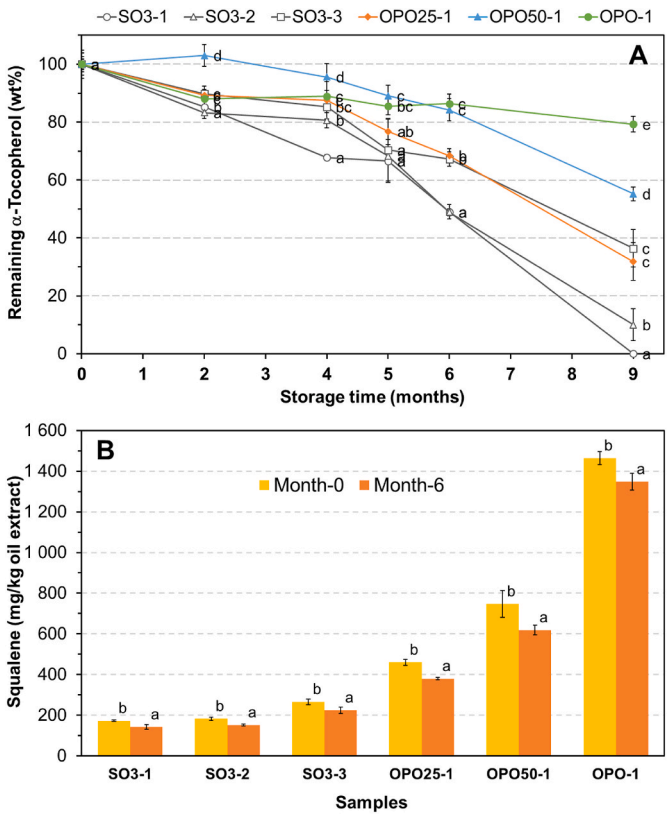

Fig. 3. Losses of α-tocopherol (**A**) and squalene (**B**) during storage. Results represent the mean and standard deviation of 3 independent cakes. Different letters in **A** indicate differences between samples according to Duncan's test ($p < 0.05$). Different letters in **B** indicate significant losses found on month-6 according to Student's *t*-test ($p < 0.05$).

nuts indicated by the consumers was considerably higher in the SO sample. Sixty percent of consumers perceived signs of rancidity in this sample after 5-month storage, while this value was considerably lower for the rest of the samples (22–33%) (Fig. S6). The fact that rancidity was indicated by consumers in OPO-1 does not contradict the sensory analysis performed by expert panellists (Fig. S4), which are trained to identify oxidative rancidity. In this regard, 5% of consumers indicated rancidity even in the fresh samples, which might have been easily confounded with other aromatic connotations.

4. Conclusions

Refined OPO has shown a high resistance to oxidative degradation during the processing and storage of traditional oil-based cakes, much higher compared to SO or blends of SO with OPO. As a consequence, a remarkable improvement in the quality of these products was found when the SO was replaced with OPO, as SO degrades readily, especially during storage. The use of high-quality refined OPO could even prolong the shelf-life of traditional oil-based cakes. In addition, the sensory perception of OPO-containing cakes by consumers has clearly proved the realistic potential of refined OPO as a food ingredient in the confectionery food sector.

In addition to a clear reduction in the levels of lipid oxidation products, the partial or total replacement of SO with OPO also enhances the nutritional properties of traditional oil-based cakes, due to the improvement in the fatty acid profile and the bioactive components of the OPO, which are not found in SO, such as triterpenic alcohols and triterpenic acids, and aliphatic alcohols, or are present in substantially lower amounts, such as squalene. These components remain at high amounts during the processing and storage.

CRediT authorship contribution statement

Joaquín Velasco: Conceptualization, Formal analysis, Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing, Supervision, Validation, Funding acquisition. **Aída García-González:** Formal analysis, Data curation, Investigation, Writing – review & editing. **Rosario Zamora:** Writing – review & editing. **Francisco J. Hidalgo:** Writing – review & editing. **M. Victoria Ruiz-Méndez:** Methodology, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.115081.

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