Olive oil aromatization with saffron by liquid–liquid extraction

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Abstract A new aromatization method for olive oils with saffron aqueous extracts rich in safranal has been developed using liquid–liquid extraction. Four flavoured olive oils were obtained (SO1–SO4). SO1 showed the highest safranal concentration (145.89 mg L⁻¹), followed by SO2 (79.33 mg L⁻¹), SO3 (0.30 mg L⁻¹) and SO4 (0.01 mg L⁻¹). Although flavouring originated a decrease in the quality parameters and the oxidative stability of the oils, even after 7 months of storage, at room and refrigeration temperatures, the oil parameters evaluated were still comparable to those of extra virgin olive oil. Flavored olive oils with less safranal (SO3, SO4) are preferred by consumers.

Keywords Oil aromatization · Safranal · Liquid–liquid extraction · Oxidative stability

Introduction

Since ancient times, spices have played an important role in cooking, cosmetics, perfumery, global exploration, economics and medicine (Dog 2006). Saffron, the dried stigmas of *Crocus sativus* L. flowers, is a good example due to its multiple applications. In addition to being the most valued spice worldwide due to its colouring properties, pleasant bitter taste and alluring aroma (Carmona et al. 2006; Ordoudi et al. 2015), saffron has a long history as a component of traditional healing. Modern medicine has also revealed its anticarcinogenic, antimutagenic, immunomodulating, neuroprotective and antioxidant-like properties (Ulbricht et al. 2011).

Saffron's colour is due to the crocetin esters, a group of water-soluble carotenoids (Serrano-Díaz et al. 2011), and a monoterpene glycoside, picrocrocin, is considered to be responsible for its bitter taste. Safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde), which results from the acid hydrolysis of picrocrocin (Fig. 1), is the major compound (approximately 70%) of the volatile fraction of saffron spice (Amanpour et al. 2015; García Rodríguez et al. 2014; Carmona et al. 2007; Tarantilis and Polissiou 1997). Numerous studies have focused on the pharmacological properties of safranal as an anxiolytic, hypnotic, antioxidant, tissue protectant, anticonvulsant, anti-inflammatory, antinociceptive, anti-cancer and memory enhancer (Ulbricht et al. 2011).

The current consumer concern about possible adverse effects of chemical preservatives, along with the growing demand for foods with a long shelf life, has resulted in significant pressure on the food industry to replace synthetic chemical additives with “natural” alternatives. This consumer-led trend has fuelled research for food preservatives derived from natural sources. Many plants are

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Fig. 2 HPLC chromatograms of saffron (a and c) and the safranal distilled extract (b and d)

Fig. 3 GC/MS chromatogram of safranal aqueous extract. (1) Acetic acid (4,99%); (2) 3,5,5-trimethyl-3-cyclohexen-2-ona (1,10%); (3) 3,7-dimethyl-1,6-octadien-3-ol (linalol) (6,68%); (4) 2,6,6-trimethyl-1,4-cyclohexadien-1- carboxaldehyde (4,35%); (5) 3,5,5-trimethyl-3-

ciclohexen-1-ona (1,94%); (6) 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde (8,38%); (7) 2,6,6- trimethyl-1,3-ciclohexadien-1- carboxaldehyde (safranal) (72,56%)

GC/MS determinations were made according to Maggi et al. (2009) as follows: ground saffron (10 mg) was placed in a stainless steel tube and dynamic headspace desorption (DHD) was carried out using a Perkin-Elmer (TurboMatrix ATD thermal desorption system, Norwalk, CT, USA). The headspace isolation conditions were as follows: oven temperature, 50 °C; desorption time, 5 min; cold trap temperature, − 30 °C; helium inlet flow rate, 45 mL min^{−1}. The desorption unit was coupled to a Varian CP-3800 gas chromatograph (Palo Alto, CA, USA) equipped with a Saturn 2200 ion trap mass spectrometer

(MS) provided with a VF-5MS Factor Four fused silica capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness) from Varian (Palo Alto, CA, USA). The column temperature was initially held for 2 min at 80 °C, then increased to 200 °C at 10 °C min^{−1} and held for 5 min and finally increased to 250 °C at 20 °C min^{−1} and held for 5 min. The transfer line and detector temperatures were set at 230 and 300 °C respectively. Helium was used as carrier gas at a flow rate 1 mL min^{−1}. In the mass spectrometer the electron impactmode was set up at 70 eV. The mass range varied from 40 to 500 u. A calibration curve was

constructed by injecting 10 μL aliquots of safranal standard solutions into stainless steel thermal tubes containing glass wool, previously conditioned for 1 min at 300 °C under a nitrogen flow of 100 mL min⁻¹.

Extra virgin olive oil from the *Arbequina* variety was used to obtain the four flavoured samples with SA extract. Healthy olive fruits were picked by hand in olive trees located in the “Campos de Hellín” area (Albacete, Spain) during the 2011/2012 crop year. The olive oil was extracted using an experimental oil mill, *Oliomio TF-30* (Toscana Enologica Mori, Tavarnelle Val di Pesa-FI-Italy), at the Escuela Técnica Superior de Ingenieros Agrónomos (ETSIA), Albacete (Castilla-La Mancha, Spain). The SO1, SO2, SO3 and SO4 flavoured olive oils were obtained by four different liquid–liquid extractions (explained later).

Two controls were also used; C0 was extra virgin olive oil from *Arbequina* variety without any treatment, C1 was *Arbequina* extra virgin olive oil subjected to liquid–liquid extraction with water acidified to a pH similar to the SA extract (pH = 3) in order to study the possible effect of acid pH on the quality parameters of the olive oil.

All samples (controls and flavoured oils) were stored in dark glass bottles at room temperature (25 °C) or refrigerated (4 °C) until analysis.

Liquid–liquid extractions of SA extract in hexane

Liquid–liquid extraction was performed on the SA extract in hexane, which was used as a reference apolar solvent. An aliquot of 450 mL of the SA extract was divided into three aliquots of 150 mL, which were deposited in three separating funnels. Three parallel extractions were carried out by adding 75 mL of hexane to each of the funnels without forming emulsions. To accelerate the safranal partition equilibrium between the polar and apolar phases, the funnels were shaken in orbital movements every 15 min, and aliquots of one mL of each phase (water and hexane) were taken for UV–vis spectrophotometric determination (“Safranal quantification”). These aliquots were returned to the dropping funnels, and the same procedure was followed for 2 h.

Liquid–liquid extractions of SA extract in olive oil

To prepare flavoured olive oils with saffron, liquid–liquid extractions were performed. An aliquot of 300 mL of the SA extract was divided into three aliquots of 100 mL deposited in three separating funnels. Three extractions were performed in parallel by adding 75 mL of olive oil to each funnel without forming emulsions. The funnels were shaken on orbital every 15 min to accelerate the partition equilibration of safranal between polar and apolar phases. The final extraction time was 1 h 45 min, followed by

15 min for decanting. The oil and water phases were separated after 2 h of liquid–liquid extraction. The olive oil samples from the three parallel extractions were pooled and transferred to dark glass bottles and stored at two different temperatures, as described in the “Samples” section, for further analyses.

Five-microlitre aliquots were used to determine safranal concentrations by UV–vis spectrophotometry and Thermal Desorption-Gas Chromatography coupled with Mass Spectrometry (TD–GC–MS), as explained in the following section. The remaining 95-mL aliquots were deposited again in the corresponding funnels, and 75-mL aliquots of olive oil were added to perform a second extraction. This same procedure was repeated until four samples of flavoured olive oil with different safranal concentrations were obtained. The sample obtained from the first liquid–liquid extraction was SO1 and SO2, SO3 and SO4 were those obtained from the second, third and fourth extraction, respectively.

Safranal quantification

UV–Vis spectrophotometry

Samples were scanned from 190 to 700 nm using a Perkin-Elmer Lambda 25 spectrophotometer (Norwalk, CT, USA) with UV WinLab 2.85.04 software (Perkin-Elmer). The absorbance at 315 nm was used to determine the safranal content in the aqueous extract and all aqueous phases from liquid–liquid extractions. However, the absorbance at 300 nm was used for determination in the hexane phases because the safranal showed the maximum absorbance at this wavelength in this solvent. All determinations were made in duplicate, and two measurements were taken for each replicate.

Quantification was carried out using two calibration curves of safranal standard solutions in the range of six levels (10.0–600.0 mg L⁻¹) in water and hexane.

Safranal concentrations in flavoured olive oil samples were determined by comparing the safranal concentrations of the SA extract before and after performing the liquid–liquid extraction.

Thermal desorption–gas chromatography–mass spectrometry (TD–GC–MS)

The aromatic fraction of the SA extract before and after liquid–liquid extraction with olive oil was extracted by stir-bar sorptive extraction (SBSE) and analyzed by GC–MS (Maggi et al. 2009). Aliquots of 100 μL of 3-methyl-1-pentanol used as the internal standard (IS) were added to one mL of the aqueous samples, and water was added to the flask to a final volume of 10 mL. A

polydimethylsiloxane-coated stir bar (0.5-mm film thickness, 10-mm length, Twister, Gerstel, Mülheim and der Ruhr, Germany) was used to stir the samples at 500 rpm at room temperature for 60 min. The stir bar was then removed from the sample, rinsed with distilled water and dried with a cellulose tissue, and later transferred into a thermal desorption tube for GC–MS analysis. Thermal desorption was performed on an ATD-400 equipment of Perkin-Elmer (Norwalk, CT). Volatile compounds were desorbed from the stir bar at the following conditions: oven temperature at 330 °C; desorption time, 4 min; cold trap temperature, – 30 °C; helium inlet flow, 45 mL min^{–1}. The compounds were transferred to a HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) and a model HP-5973 mass spectrometer with a fused silica capillary column (BP21 stationary phase, 50-m length, 0.22 mm i.d., 0.25 µm film thickness; SGE, Ringwood, Australia). The chromatographic program was set at 40 °C (held for 2 min), raised to 150 °C at 10 °C min^{–1} (held for 5 min) and then raised to 230 °C at 10 °C min^{–1} (held for 2 min). The total analysis time was 28 min. For mass spectrometry analysis, electron impact mode (EI) at 70 eV was used. The mass range varied from 35 to 500 µ and the detector temperature was 150 °C. Determinations were made in duplicate, and because the liquid–liquid extractions were performed in triplicate, the results were the mean of six analyses. Identification was carried out using the NIST library and by comparison with the mass spectrum and retention index of chromatographic standards we designed. To avoid matrix interference, the MS quantification was carried out using single-ion monitoring (SIM). The safranal concentrations of the SA extract and aqueous phases of the liquid–liquid extractions were quantified based on six-point calibration curves ($R^2 > 0.97$) of 0.4–131 mg L^{–1} of an aqueous solution of the safranal standard.

Safranal concentrations in flavoured olive oil samples were obtained by comparing the safranal concentrations of the SA extract before and after performing the liquid–liquid extraction.

Quality indices of olive oils

Quality indices were measured in the same way as in Pardo et al. (2013). Determination of the regulated physico-chemical quality parameters (free acidity, peroxide value and UV absorption characteristic, K_{270} and K_{232}) was carried out according to the analytical methods recommended and described by Regulation EEC/2568/91 of the Commission of the European Union (EEC 1991).

Free acidity, expressed as the percentage of free oleic acid, was determined by the titration of a solution of oil

dissolved in ethanol/ether (1:1) with 0.1 M potassium hydroxide ethanolic solution.

Peroxide value, expressed in milliequivalents of active oxygen per kilogram of oil (meq/kg), was determined as follows: a mixture of oil and chloroform–acetic acid was left to react with a solution of potassium iodide in darkness; the free iodine was then titrated with a sodium thiosulfate solution.

The K_{270} and K_{232} extinction coefficients were calculated from absorption at 270 and 232 nm, respectively, with an UV spectrophotometer (Hewlett-Packard, HP 8452 A), using a 1% solution of oil in cyclohexane and a path length of 1 cm.

Shelf life prediction

Oxidative stability was evaluated by the Rancimat method (Gutiérrez 1989). Stability was expressed as the oxidation induction time (hours) measured with the Rancimat 679 apparatus (Metrohm Co., Basel, Switzerland) using an oil sample of 3.5 g warmed to 100 °C and air flow of 10 L/h. To evaluate the oxidative stability of the olive oil samples during storage, samples were analyzed at production time and monthly over 7 months of storage.

Sensorial evaluation

Sensory assessment of different olive oil samples (flavoured and C0) was performed on fresh olive oil samples (day 0 of storage) and was carried out by 105 consumer-type panellists, selected on the condition that they were regular consumers, aged between 15 and 70 years, and with no distinction regarding gender. The samples were labelled, and the tasters were asked to smell and taste. The samples were supplied at a temperature of 28 ± 2 °C in blue coloured glasses. The tasters ranked the samples by their degree of smell and flavour acceptability using a descriptive hedonic scale included within affective test (Anzaldúa 1994). Each hedonic description was assigned a nine-point scale (– 4 = dislike extremely, 0 = neither like nor dislike, 4 = like extremely) (Peryam and Girardot 1952). Each parameter was analyzed separately. The mean scores for both sensory properties were calculated.

Statistical analysis

Analytical determinations were performed at least in duplicate, and the data were processed by SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Significant differences among means were determined by an analysis of variance (ANOVA) using Duncan's test with a 95% significant level ($p < 0.05$). ANOVA was used to evaluate the effect of the different liquid–liquid extractions of SA extract on the

quality indices, oxidative stability and acceptability of olive oils.

Results and discussion

The characterization of saffron and the safranal enriched extract are shown in Figs. 2 and 3. The chromatograms of the saffron extract obtained by HPLC at 250 and 330 nm are shown in Fig. 2a, c. These are the chromatographic cycles typical of saffron samples of the DOP “Azafrán de la Mancha”. The chromatograms obtained by HPLC at the same wavelengths of the distillate are also shown in Fig. 2b, d. Figure 3 shows the chromatogram obtained from the starting saffron by TD–GC–MS, which presents the typical profile of the chromatograms obtained from samples of saffron of the DOP “Azafrán de la Mancha”.

Liquid–liquid extraction conditions

The safranal distribution was studied in a model system of polar–apolar phases (Fig. 4) to establish the partition equilibrium of safranal, the compound that most contributes to saffron aroma (Carmona et al. 2007; Tarantilis and Polissiou 1997). Figure 4 shows how safranal dissolved in water (polar phase) shifted to the apolar phase (hexane). After 90 min, which is the time when partition equilibrium of safranal was achieved, the concentration of safranal in water was near 0. This experiment was used to set the time for the liquid–liquid extractions to produce all flavoured olive oils. To ensure the total transfer of safranal to the olive oil, the duration of the liquid–liquid extractions between the SA extract and olive oil samples was set at 120 min (more than 90 min) because olive oil is a more complex matrix than the hexane apolar solvent.

Safranal concentrations in flavoured olive oils

The elaboration of an olive oil with aromatic notes of saffron by maceration is difficult due to the low safranal content of the saffron spice (lower than 0.25% w/w) according to Maggi et al. (2011). Therefore, in this study, aqueous saffron extract rich in safranal was prepared (Table 1) by liquid–liquid extraction. The SA extract was not changed in each of the four different extractions because the objective was to obtain flavoured olive oils with different safranal concentrations. Thus, extractions were made consecutively by adding a new olive oil sample to the used SA extract. As shown in Table 1, the flavoured olive oils from the first extraction (SO1) showed the highest safranal concentrations. The concentrations obtained by UV–vis spectrophotometry were slightly higher than those obtained by TD–GC–MS, which may be due to interference from other compounds different from the safranal detected by UV–vis spectrophotometry. However, the TD–GC–MS chromatographic technique is capable of separating the compounds without producing overlaps in the safranal chromatographic peak. Flavoured olive oils obtained from the second extraction (SO2) also showed high concentrations of safranal (79.33 mg L⁻¹) compared with those obtained from the third and fourth extraction (SO3 and SO4), whose safranal concentrations were 0.30 and 0.01 mg L⁻¹, respectively.

Quality parameters of flavoured olive oils

Table 2 presents the mean values of the physicochemical quality parameters for the controls and flavoured olive oils at production and after 7 months of storage. At the beginning of storage, all samples showed lower values than the limits set by Regulation EC/1989/2003 (EC 2003) for

Fig. 4 Safranal distribution in water and hexane after 2 h of liquid–liquid extraction of 150 mL of the SA extract (265 mg L⁻¹) in 75 mL of hexane

Table 1 Safranal concentration (mg L⁻¹) by thermal desorption-gas chromatography-mass spectrometry (TD–GC–MS) of the original saffron aqueous extract rich in safranal (SA extract) and of aqueous phase (water) and oil phase (olive oil) after four liquid–liquid extractions (2 h) of 100 mL of the SA extract in 75 mL of *Arbequina* virgin olive oil samples

	Safranal by TD–GC–MS	
	Aqueous phase	Oil phase
SA extract	169.24	
First extraction	59.82	145.89
Second extraction	0.32	79.33
Third extraction	0.10	0.30
Fourth extraction	0.09	0.01

the extra virgin category (free acidity $\leq 0.8^\circ$; peroxide index ≤ 20 meq O₂/kg; K₂₇₀ ≤ 0.22 ; K₂₃₂ ≤ 2.5). The aromatization process with a SA extract caused a slight increase in free acidity, peroxide index and extinction

coefficients (K₂₇₀ and K₂₃₂). Thus, peroxide value was higher in flavoured olive oils, independent of the safranal concentration, without exceeding the limits for extra virgin olive oil. The flavoured olive oils with the highest safranal concentration also stood out for the highest K₂₃₂ coefficient but always remained well below the limits set forth for an extra virgin olive oil. Table 2 also illustrates the results of these quality parameters during the storage at room and refrigeration temperatures. In all cases, the parameter values were low except for the peroxide value in some samples. No significant differences in free acidity were found between flavoured oils and the controls after 7 months of storage. This demonstrates that the safranal did not affect acidity. After that time, peroxide index also increased in all samples, even the flavoured olive oils stored at room temperature, exceeding the limit fixed by the European regulation for extra virgin olive oil. The oxidation products, evaluated through K₂₇₀ and K₂₃₂, increased in all samples during storage, with a higher increase at room temperature, although they remained below the limit for

Table 2 Mean values of the physicochemical quality parameters for the controls and flavored olive oils with saffron aqueous extract rich in safranal at production and 7 months of storage

	Free acidity (%)			Peroxide index (meqO ₂ /kg)		
	At production	After 7 months		At production	After 7 months	
		Room	4 °C		Room	4 °C
C0	0.11 ± 0.06	0.13 ± 0.02	0.11 ± 0.04	9.00 ± 1.02 ^b	15.00 ± 1.05 ^x	12.50 ± 1.02 ^{ab}
C1	0.06 ± 0.01	0.14 ± 0.02	0.09 ± 0.02	10.50 ± 1.03 ^b	16.50 ± 1.08 ^x	15.50 ± 1.01 ^{ab}
SO1	0.09 ± 0.06	0.19 ± 0.03	0.13 ± 0.03	13.00 ± 1.05 ^a	> 25*	17.00 ± 1.02 ^a
SO2	0.11 ± 0.11	0.17 ± 0.05	0.14 ± 0.04	13.00 ± 1.08 ^a	> 25*	17.50 ± 1.03 ^a
SO3	0.08 ± 0.05	0.16 ± 0.05	0.16 ± 0.05	13.00 ± 1.10 ^a	> 25*	16.00 ± 1.03 ^a
SO4	0.07 ± 0.05	0.17 ± 0.04	0.12 ± 0.06	12.50 ± 1.02 ^a	> 25*	14.00 ± 1.05 ^a
K₂₇₀						
	At production	After 7 months		At production	After 7 months	
		Room	4 °C		Room	4 °C
C0	0.08 ± 0.01 ^y	0.17 ± 0.01 ^x	0.09 ± 0.01 ^y	1.19 ± 0.05 ^{b,y}	1.55 ± 0.01 ^{b,x}	1.26 ± 0.06 ^{b,y}
C1	0.03 ± 0.01 ^y	0.13 ± 0.01 ^x	0.07 ± 0.01 ^{xy}	1.10 ± 0.01 ^{b,y}	1.53 ± 0.02 ^{b,x}	1.20 ± 0.05 ^{b,y}
SO1	0.08 ± 0.01 ^y	0.18 ± 0.01 ^x	0.12 ± 0.05 ^{xy}	1.60 ± 0.07 ^{a,y}	2.32 ± 0.02 ^{a,x}	1.83 ± 0.05 ^{a,y}
SO2	0.10 ± 0.01 ^y	0.15 ± 0.01 ^x	0.12 ± 0.03 ^{xy}	1.48 ± 0.05 ^{a,y}	2.27 ± 0.03 ^{a,x}	1.52 ± 0.07 ^{a,y}
SO3	0.02 ± 0.01 ^y	0.15 ± 0.01 ^x	0.05 ± 0.01 ^y	1.21 ± 0.03 ^{b,y}	2.24 ± 0.03 ^{a,x}	1.31 ± 0.06 ^{b,y}
SO4	0.05 ± 0.01 ^y	0.19 ± 0.01 ^x	0.07 ± 0.01 ^y	1.27 ± 0.06 ^{b,y}	2.33 ± 0.04 ^{a,x}	1.39 ± 0.05 ^{b,y}

C0 Control 0 (*Arbequina* extra virgin olive oil); C1 Control 1 (C0 subjected to a liquid–liquid extraction with water at pH 3); SO1 flavored olive oil with SA extract after the first liquid–liquid extraction; SO2 flavored olive oil with SA extract after the second liquid–liquid extraction; SO3 flavored olive oil with SA extract after the third liquid–liquid extraction; SO4 flavored olive oil with SA extract after the fourth liquid–liquid extraction

*The maximum limit of quantification is exceeded

^{a,b}In columns, different superscripts for the same quality parameter mean significant differences among samples

^{x,y}In lines, different superscripts for the same quality parameter mean significant differences among temperatures storage

extra virgin olive oils. No significant differences in the K_{270} coefficient were found between flavoured oils and the controls for both temperatures. In contrast, with respect to K_{232} , the flavoured olive oils with higher concentrations of safranal stood out for their higher values after storage at four degrees, and significant differences were found between the flavoured oils and controls at the room temperature. Very similar behaviour of these quality parameters was found in olive oils flavoured with other spices and herbs stored at room temperature and aromatized by maceration (Baiano et al. 2009; Gambacorta et al. 2007).

Evolution of oxidative stability

Saffron is an antioxidant source that can be used as functional ingredient (Serrano-Díaz et al. 2012). It has been demonstrated to have some protective effects on different markers of oxidative damage in hippocampal tissue from ischemic rats (Hosseinzadeh and Sadeghnia 2005) and to improve chemically induced diabetes and its complications by modulation of oxidative stress (Samarghandian et al. 2013). *Arbequina* oils are characterized by their low oxidative stability compared to other Spanish varieties (Pardo et al. 2013). To study the possible effect of safranal on the shelf life of virgin olive oils, two storage tests were performed at different temperatures. Figure 5 shows the evolution of the oxidative stability of the controls and flavoured olive oils with different concentration of safranal during storage at room (Fig. 5a) and refrigeration (Fig. 5b) temperatures. At production, the oxidative stability of the samples was low, as expected for *Arbequina* oils with a low polyphenol content, with values below 50 h (Pardo et al. 2013). In addition, the highest oxidative stability was detected in the controls and was significantly different from that of the flavoured samples. These differences could be due to the following two facts, (1) the transference of safranal to the oil during the liquid–liquid extraction process and (2) the transfer of phenolic compounds from the oil to SA extract, because these compounds are partially soluble in water. During storage at room temperature, a decrease in the oxidative stability of the controls and flavoured olive oils was noted. The evolution of oxidative stability in the controls oils was linear (C0: $y = -4.1652x + 51.295$, $R^2 = 0.991$; C1: $y = -4.11x + 47.101$, $R^2 = 0.993$), while the trend of flavoured oils was fit an exponential curve, following a first order kinetics as they were of the type $y = A_0 * e^{-\lambda * t}$ (SO1: $y = -2870.09 * e^{-5.3901x}$, $R^2 = 0.97$; SO2: $y = -1518.84 * e^{-5.0407x}$, $R^2 = 0.97$; SO3: $y = -3906.90 * e^{-5.71x}$, $R^2 = 0.96$; SO4: $y = -1220.72 * e^{-4.8231x}$, $R^2 = 0.96$). Thus, a sharp decrease in the oxidative stability of the flavoured oils was observed after the first month of storage, an oxidative

stability loss of approximately 51%. After 7 months, the oxidative stability loss was approximately 97% for the flavoured oils, and the controls oils remained more stable, with a loss of approximately 66%. In contrast, during refrigerated storage, the oxidative stability of all samples decreased very slowly. In the controls, the oxidative stability remained practically constant, and in the case of flavoured oils, the decline was only 8%. Therefore, when the flavoured oils are stored at room temperature, we can say that safranal does not extend the shelf life of olive oils, as occurs with the addition of other spices and herbs (Asensio et al. 2013; Ayadi et al. 2009; Baiano et al. 2009; Gambacorta et al. 2007). Despite the fact that the oxidative stability of flavoured oils decreases during storage at room temperature, it is noteworthy that they remain stable for a long time when the oils are stored at 4 °C. Thus, conservation under refrigeration at 4 °C may help to increase shelf-life of flavoured oils.

Consumer acceptability study

The consumer acceptability of flavoured olive oils with SA extract is important for the introduction of this new product to the oil market. Figure 6 shows the consumer acceptance of the flavoured olive oils. The control used in tasting was C0, consisting in extra virgin olive oil from the *Arbequina* variety. Significant differences in the smell between flavoured oils and the control were found, suggesting that this flavouring method provides aromas of saffron to the oil. However, the odor of the control was valued higher than the other samples, probably due to the appearance of strange aromas originated by the addition of safranal, which can be considered as defects by the regular consumers of olive oil. Within the flavoured oils, those with the lower concentration of safranal (SO4) was the most accepted with values over 1, which indicates that these oils like to consumers. As regards taste, a similar pattern was observed. Panellists showed greater satisfaction with the control taste, although no significant differences were found for the flavoured olive oils with lower concentration of safranal (SO3 and SO4). In this case, the higher concentrations of safranal were evaluated negatively, indicating that safranal in higher concentrations provides a strong flavour to oil that consumers do not like. According to these results, a section of the population may welcome the smell and taste of safranal in olive oil when lower concentrations are used for the aromatization. The degree of satisfaction decreases when the safranal concentration is used in higher concentrations. These findings highlight the importance of optimizing concentrations when strong spices are used, and suggest the opportunity to aromatize olive oils with smaller concentrations of saffron.

Fig. 5 Trend of the oxidative stability during storage of the controls and flavoured olive oils with saffron aqueous extract rich in safranal by four liquid–liquid extractions: **a** at room temperature and **b** at 4 °C. C0: Control 0 (*Arbequina* extra virgin olive oil); C1: Control 1 (C0 subjected to a liquid–liquid extraction with water at pH 3); SO1: Flavoured olive oil with SA extract after the first liquid–liquid extraction; SO2: Flavoured olive oil with SA extract after the second liquid–liquid extraction; SO3: Flavoured olive oil with SA extract after the third liquid–liquid extraction; SO4: Flavoured olive oil with SA extract after the fourth liquid–liquid extraction

The letter on the bar means that there are significant differences among samples for each attribute ($p < 0.05$).

Fig. 6 Consumer acceptance (9-point hedonic scale) the flavoured olive oils with saffron aqueous extract rich in safranal by four liquid–liquid extractions. C0: Control 0 (*Arbequina* extra virgin olive oil) SO1: Flavoured olive oil with SA extract after the first liquid–liquid

extraction; SO2: Flavoured olive oil with SA extract after the second liquid–liquid extraction; SO3: Flavoured olive oil with SA extract after the third liquid–liquid extraction; SO4: Flavoured olive oil with SA extract after the fourth liquid–liquid extraction

Conclusion

A method of flavouring of olive oil with saffron has been developed using liquid–liquid extraction of a saffron aqueous extract rich in safranal. The aromatization process with SA extract did not alter the characteristic values of extra virgin category of *Arbequina* oil used in the elaboration of the flavoured samples. Although the controls had higher values of oxidative stability, the flavoured samples showed positive values of stability typical of the variety. The patterns of oxidation between the controls and flavoured olive oils during storage at room temperature shows that the optimum conservation temperature of olive oils flavoured with SA extract is 4 °C. Flavoured oils with small concentrations of safranal (SO4) were organoleptically more valued by the consumers, which make their introduction in the delicatessen market possible. Finally, this rapid and simple method of flavouring could be used to flavour other types of oil, even with different aqueous extracts.

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