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Changes in the oxidative state of extra virgin olive oil used in baked Italian *focaccia* topped with different ingredients

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Abstract

Four different types of "*focaccia*" (Italian flat bread) prepared with the same dough and the same extra virgin olive oil but with different seasonings, were analyzed. Lipids were extracted from each sample using the Folch method. The indices commonly used to assess oil quality, the amounts of *trans* fatty acids and compounds of triglyceride polymerization, oxidation and hydrolysis, were determined in all the samples to better assess the degree of oxidation and hydrolysis of the oils. The findings showed that, once baked, the oil sampled from the different types of *focaccias* could not be included in the virgin category. The level of oxidation of the baked samples was greater than that in the uncooked oil. However the results obtained showed that the level of degradation of the extracted oils was lower than that found in edible refined oils and it seemed to be influenced by the topping used to flavour the *focaccias*.

Keywords: Extra virgin olive oil; *Focaccia*; Oxidized triglycerides; Triglyceride oligopolymers

1. Introduction

Focaccia is a very popular baked bread product, common to many Italian regions. It is made from regular bread dough rolled out into varying thicknesses, seasoned with salt and oil and baked in a low pan. Whether with simple or richer toppings, it is often used as a tasty alternative to bread.

Baked products have been studied extensively. Investigations have specifically regarded the rheological properties of dough, the structural properties of finished products, their shelf-life, the thermomechanical features of the baking process, and the phenomena causing staleness (Azizi, Rajabzadeh, & Riahi, 2003; Bárcenas & Rosell, 2005; Dobraszczyk & Morgenstern, 2003; Goesaert et al., 2005; Patel, Waniska, & Seetharaman, 2005; Raffo et al., 2003). With the exception of some investigations regarding *trans* fatty acids, little research has focussed on the impact of baking on the oxidative state of the lipid fraction of these products (Priego-Capote, Ruiz-Jiménez, García-Olmo, & Luque De Castro, 2004; Quílez, Ruiz, Brufau, & Rafecas, 2006; van Erp-baart et al., 1998). Oxidative phenomena, regarding lipids, have been studied, essentially for other cooking methods, such as frying (Coni, Podestà, & Catone, 2004; Gil, Cho, & Yoon, 2004; Naz, Sheikh, Siddiqi, & Asad Sayeed, 2004; Naz, Siddiqi, Sheikh, & Sayeed, 2005; Quiles, Ramírez-Tortosa, Gómez, Huertas, & Mataix, 2002; Ramírez & Cava, 2005; Zhang, Wu, & Weng, 2004). The aim of this investigation was to collect experimental data to evaluate the level of oxidation of oil used in baking *focaccias* topped with a variety of ingredients and cooked in non-industrial ovens and to determine the quality characteristics of this important Italian product.

2. Materials and methods

Four different types of *focaccia* made from the same dough, topped with different ingredients, but flavoured with the same extra virgin olive oil, were studied. The

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focaccias were baked in a thermoventilated gas oven with four baking chambers at 220 °C for 20 min.

The four types were as follows:

– Tomato-topped *focaccia*: about 400 g of dough topped with 200 g of small chunks of raw tomatoes (moisture: 94%).
– Potato-topped *focaccia*: about 400 g of dough topped with 250 g of diced raw potatoes (moisture: 78.5%).
– Rosemary-topped *focaccia*: about 400 g of dough topped with 2 g of dry rosemary.
– Onion-topped *focaccia*: about 400 g of dough topped with 200 g of sliced raw onions (moisture: 92.1%).

After baking, the diameter of the *focaccias* was 26–27 cm and their thickness was 1.8–2.2 cm.

Once in the laboratory, the oil was cold extracted from each *focaccia* with the method described by Folch, Lees, and Sloane-Stanley (1957) and kept in a freezer at −20 °C until analyzed. Routine analyses were performed on the raw oil, as well as the baked oil samples, to determine percent free fatty acids (FFA), peroxide value (PV) and UV absorption (Official Journal of the European Communities, 1991) and to measure the *p*-anisidine value (*p*-AV) (IUPAC, 1987a).

The acidic composition of the oil was determined as indicated by EC regulation no. 2568/91 (Official Journal of the European Communities, 1991). The gas chromatograph used was a Fisons HRGC Mega 2 Series (Milan, Italy), with a flame ionization detector, equipped with a WCOT fused silica capillary column from Chrompack, (Varian, Inc., Palo Alto, CA, USA), FFAP-CB coating, 25 m in length × 0.32 mm i.d., and film thickness 0.30 μm. The oven temperature was isothermal at 180 °C, while the temperature of the split injector was 270 °C with a split ratio of 1:17, and detector temperature was 300 °C. The carrier gas was hydrogen at a flow rate of 2 ml/min.

Unconventional analytical parameters were also examined to better evaluate the degrees of oxidative and hydrolytic degradation of the oils. Silica gel chromatography was performed according to the IUPAC method (1987b) to separate polar compounds (PC) from the oils and high performance size-exclusion chromatography (HPSEC) of the PC, to determine the triglyceride oligopolymers (TGP), oxidized triglycerides (ox-TG), and diglycerides (DG). The HPSEC system consisted of a series 200 pump (Perkin–Elmer) with a 7125 S sample injector, 50 μl injector loop, a PL-gel guard column (5 cm × 0.75 cm i.d., Perkin–Elmer) and a series of two PL-gel columns (30 cm × 0.75 cm i.d., Perkin–Elmer) packed with highly cross-linked styrene divinylbenzene copolymer with a particle diameter of 5 μm and pore diameters of 500 Å. The elution solvent used was CH_2Cl_2 for HPLC at a flow rate of 1 ml/min. A differential refractometer (series 200, Perkin–Elmer) was utilized as detector. Peak identifications and quantification were carried out as described elsewhere (Gomes, 1992; Gomes & Caponio, 1999).

The fatty acid *trans* isomers were determined as described in EC Regulation no. 1429/92 (Official Journal of the European Communities, 1992), utilizing a HRGC Mega 2 series (Milan, Italy) gas-chromatograph with a flame ionization detector, equipped with a SPTM 2340 fused silica capillary column (60 m in length × 0.25 mm i.d. and film thickness 0.20 μm, Supelco). Temperature was set to range from 160 to 200 °C with increases of 1.3 °C/min; temperature of the split injector was 210 °C with a split ratio of 1:100, and detector temperature was 220 °C. The carrier gas was hydrogen.

The data obtained were compared by variance analysis (ANOVA), followed by the multiple range test, with the Statgraphics Plus software (Manugistics, Rockville, USA).

3. Results and discussion

The acidic composition of the extra virgin olive oil used to make the *focaccias* is shown in Table 1.

The results of the routine analyses performed on the raw oil and on the baked oil samples (Table 2) provided initial information about the hydrolytic and oxidative degradation that occurred during baking of the *focaccias*. The percent free fatty acids increased substantially in the tomato-topped *focaccia,* with values that were about 3-fold greater than in the raw oil. This was probably ascribable to the acidic nature of the ingredient used as a seasoning. The levels of hydrolytic degradation remained below the 2% threshold value prescribed by the European legislation for virgin olive oils (Official Journal of the European Communities, 2003).

By contrast, the indices of oxidative degradation pointed to a worsening of the quality of the oils extracted from the baked *focaccias,* with values that were beyond those of virgin olive oils. The peroxide value of the raw extra virgin olive oil used in the investigation was 16.4 meq/kg (hence, within the legal limit of 20 meq/kg), the oils extracted after baking had substantially greater values, ranging from 26.3 meq/kg to 57.6 meq/kg which were

Table 1
Percent acidic composition of the extra virgin olive oil employed in the study

Fatty acid	Mean[a] (%) ± SD
$C_{14:0}$	0.05 ± 0.01
$C_{16:0}$	11.1 ± 0.28
$C_{16:1}$	0.73 ± 0.01
$C_{17:0}$	0.05 ± 0.01
$C_{17:1}$	0.08 ± 0.00
$C_{18:0}$	2.90 ± 0.02
$C_{18:1}$	78.0 ± 0.05
$C_{18:2}$	5.89 ± 0.14
$C_{18:3}$	0.58 ± 0.01
$C_{20:0}$	tr.
$C_{20:1}$	0.34 ± 0.02
$C_{22:0}$	0.19 ± 0.01
$C_{24:0}$	0.18 ± 0.01

[a] Mean values of two independent repetitions.

Table 2
Mean results of the routine analyses for each oil sample examined[a]

Samples	FFA (%)	PV (meq/kg)	p-AV (g^{-1})	K_{232}	K_{270}	ΔK	TOTOX
Extra virgin olive oil	0.6	16.4	5.9	2.110	0.136	−0.021	38.7
Oil extracted from							
Potato-topped focaccia	1.0	30.7	16.6	2.386	0.734	0.004	78.0
Tomato-topped focaccia	1.9	26.3	32.8	2.396	1.041	0.025	85.4
Onion-topped focaccia	0.7	51.7	22.4	2.121	0.655	0.011	126
Rosemary-topped focaccia	1.1	57.6	12.0	2.217	0.680	0.011	127

[a] Mean values of two independent repetitions. FFA, free fatty acids; PV, peroxide value; p-AV, p-anisidine value; K_{232}, specific absorbance at 232 nm; K_{270}, specific absorbance at 270 nm; $\Delta K = K_{270} - (K_{266} + K_{274})/2$; and TOTOX = 2PV + p-AV.

all above the legal limit. The p-AV test results indicated that extensive secondary oxidative degradation had occurred since the p-AV determinations of the baked oils were at least almost twofold greater than the values of the raw oil. K_{270} increased dramatically after baking from 0.136 (legal limit: 0.22) in the unbaked oil to values ranging from 0.655 to 1.041 whereas K_{232} and ΔK showed only slight increases. Total oxidation of the baked oils, evaluated as TOTOX (2PV + pAV) was 2- to 3-fold greater than that in the unbaked oil, with especially higher values in the oils extracted from the *focaccias* topped with onions and rosemary.

HPSEC of the PC provided more detailed information on the oxidative and hydrolytic degradation of the baked oil samples via the determination of the following classes of compounds: triglyceride oligopolymers (TGP), oxidized triglycerides (ox-TG) and diglycerides (DG). The HPSEC chromatograms of the polar compounds of the unbaked oil, and of the same oil sampled after baking the onion-topped *focaccia,* are shown in Fig. 1. As already shown by the routine analyses, the raw oil already contained detectable amounts of TGP that were indicative of advanced oxidation. Once it was sampled after *focaccia* baking the same oil contained substantially higher TGP and ox-TG levels.

The PC data, and the results of the HPSEC analyses of the PCs of the oils examined, are shown in Table 3, together with the statistical data. PCs define the extent of the overall degradation of an oil since they include classes of substances of triglyceride oxidation, polymerization and hydrolysis. The amount of PCs in the baked oils was significantly greater in all the samples than it was in the uncooked extra virgin olive oil. The smallest difference was observed in the potato-topped *focaccia* (percent increase of 10% over the uncooked oil). In the oil samples from the other 3 types of *focaccia,* substantial increases in the PC ranged from 43% to 66%. The percent amount of ox-TG measured in the uncooked oil was 0.75%, which was not statistically different from the amount measured in the oil sampled from the potato-topped *focaccia*. By contrast, statistically significant differences were registered in the oil samples from the other types of *focaccia,* with ox-TG values ranging from 1.4 to 2.7 times the amount measured in the uncooked oil. These findings confirm that the oils sampled from the *focaccias* topped with tomatoes,

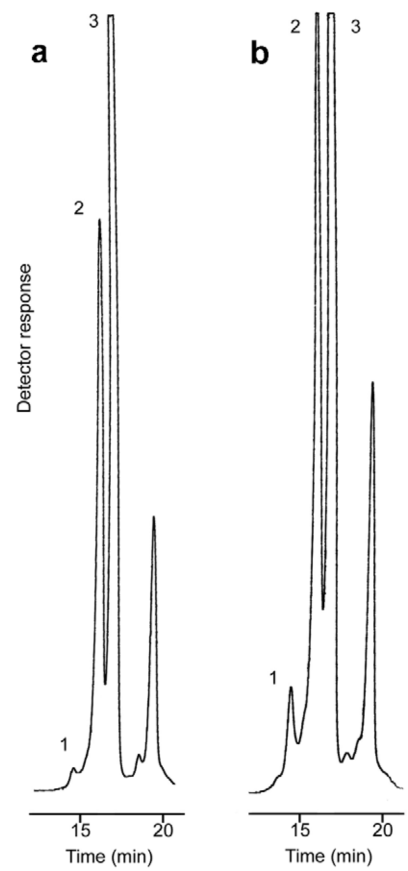

Fig. 1. HPSEC chromatograms of the polar compounds of the unbaked oil (a) and of the same oil sampled after baking the onion-topped *focaccia* (b). 1, Triglyceride oligopolymers; 2, oxidized triglycerides; and 3, diglycerides.

onions and rosemary had undergone more intense oxidation, as already highlighted by the TOTOX values in Table 2.

Baking led to a significant increase in the percentages of oligopolymers in all the oils sampled from the baked *focaccias*. The amount of TGP in the uncooked oil was 0.08%. In the potato-topped *focaccia* it was 0.13%, i.e. over 1.5 times that of the uncooked oil while, in the other three oils sampled, the amount of TGP was almost threefold that of the raw oil. Evaluation of the oligopolymers provided further evidence that the oil extracted from the potato-topped *focaccia* presented a less intense degradation than did the other baked oils.

Table 3
Percentage values (w/w) in oil of the polar compounds and of the main substance classes constituting them for each oil sample examined[a]

Samples	PC	TGP	ox-TG	DG	2TGP + ox-TG
Extra virgin olive oil	3.34 ± 0.10a	0.08 ± 0.01a	0.75 ± 0.04a	1.63 ± 0.16a	0.91 ± 0.04a
Oil extracted from					
Potato-topped focaccia	3.68 ± 0.06b	0.13 ± 0.03b	0.73 ± 0.05a	1.55 ± 0.01a	0.99 ± 0.06b
Tomato-topped focaccia	5.44 ± 0.10c	0.22 ± 0.03c	1.06 ± 0.13b	1.95 ± 0.05b	1.50 ± 0.12c
Onion-topped focaccia	4.78 ± 0.12d	0.27 ± 0.02d	1.69 ± 0.19c	1.83 ± 0.06b	2.23 ± 0.15d
Rosemary-topped focaccia	5.55 ± 0.12c	0.25 ± 0.03c	2.08 ± 0.32d	1.85 ± 0.05b	2.58 ± 0.31d

[a] Results of statistical analysis at $p < 0.05$. Mean values of three independent repetitions ± SD; one common letter following an entry indicates no significance. PC, polar compounds; TGP, triglyceride polymers; ox-TG, oxidized triglyderides; and DG, diglycerides.

The percentage of diglycerides in the uncooked oil was 1.63%. Significantly higher values were found in the baked oils, except for the oil sampled from the potato-topped *focaccia*. Determination of DG, coupled with that of percent free fatty acids, provides a better evaluation of the hydrolytic degradation of the oil. Finally, Table 3 shows the mean values and SD of the sum (2TGP% + ox-TG%), a parameter which provides a better evaluation of the overall oxidation of oil, as already described in a previous paper (Gomes, Caponio, & Delcuratolo, 2003). Substantial increases in overall oxidative degradation were found after baking in the *focaccia* oil. Once again, the oil from the potato-topped *focaccia* seemed to be less affected by the baking process with an overall oxidation index (2TGP% + ox-TG%) of 0.99 as compared to 0.91, which had been measured in the uncooked oil. As already shown with the total oxidation index (TOTOX), the oils from the *focaccias* topped with onions and rosemary proved to be the most oxidized.

The different levels of oxidation found in the oils sampled from the different types of *focaccias* seem to be ascribable to the amounts of the toppings used, their properties and their percent humidity. During baking, greater quantities of more humid toppings have the effect of stemming the rise in temperature in the *focaccia* due to the evaporation of water, thus exposing the oil to less intense thermal stress. Hence, seasoning *focaccia* with diced potatoes seemed to have better protected the oil from oxidation during baking.

When dry rosemary was used as a seasoning, the amount used was only 2 g, sprinkled over the entire surface of the *focaccia*. In this case, no evaporation took place during cooking to promote a protective effect. Although it is known that rosemary has anti-oxidant activities, the oil sampled from the rosemary-topped *focaccia* presented a greater level of degradation.

Given the substantial concordance between the two indices, TOTOX and (2TGP% + ox-TG%), we explored whether there was any positive correlation between them. Fig. 2 plots (2TGP% + ox-TG%) as a function of TOTOX for all the oil samples analyzed. As can be observed, a positive correlation between the two indices was found with $p < 0.05$.

Finally Table 4 contains the mean percent amounts of *trans* isomers of fatty acids and the results of the statistical analyses. The uncooked oil had 0.022% of $C_{18:1 trans}$, which was considerably lower than the legal limit (0.05%) and

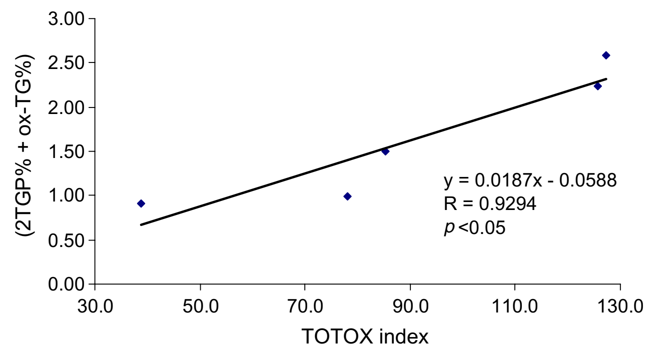

Fig. 2. (2TGP% + ox-TG%) plotted against TOTOX (2PV + *p*-AV) for all the oil samples.

contained trace levels of *trans* isomers of linoleic and linolenic acids. The baked oils extracted from the *focaccias* had amounts of $C_{18:1 trans}$ that were 4–5 times greater than the levels in the uncooked oil and the differences were statistically significant. The levels of *trans* isomers of linoleic and linolenic acids in the baked oils ranged from 0.008% to 0.023% without exceeding the legal limit for virgin olive oils (0.05%). The oil sampled from the rosemary-topped *focaccia* had lower levels of both $C_{18:1 trans}$ and *trans* isomers of linoleic and linolenic acids.

When the results of the analyses performed on the baked *focaccia* oils were compared with those of analyses on other food products, the overall level of oxidation, expressed in terms of (2TGP% + ox-TG%), proved to be lower than the levels found in refined oils (Gomes & Caponio, 1997; Gomes et al., 2003) and considerably lower than the levels found in

Table 4
Percentage values (w/w) in oil of the *trans* isomers of fatty acids for each oil sample examined[a]

Samples	$C_{18:1t}$	$C_{18:2t} + C_{18:3t}$
Extra virgin olive oil	0.022 ± 0.002a	tr.a
Oil extracted from		
Potato-topped focaccia	0.110 ± 0.018bc	0.023 ± 0.003b
Tomato-topped focaccia	0.105 ± 0.006bc	0.012 ± 0.001c
Onion-topped focaccia	0.125 ± 0.007b	0.010 ± 0.001c
Rosemary-topped focaccia	0.085 ± 0.008c	0.008 ± 0.004c

[a] Results of statistical analysis at $p < 0.05$. Mean values of three independent repetitions ± SD; one common letter following an entry indicates no significance. tr., traces (not integrated).

oils cooked by different methods, especially fried oils (Arroyo, Cuesta, Garrido-Polonio, López-Varela, & Sánchez-Muniz, 1992; Keijbets, Ebbenhorst-Seller, & Ruisch, 1986; Sebedio, Grandgirard, Septier, & Prevost, 1987).

Also, the amounts of *trans* isomers of fatty acids found in the present investigation were smaller than the amounts generally reported for other baked products or breads (van Erp-baart et al., 1998).

In conclusion, the *focaccia* baking procedure causes relatively small oxidation of the oil used for flavouring. It is better to bake *focaccias* with superior grade oils, such as extra virgin olive oil, which appears to be particularly resistant to thermal oxidation, thanks to the presence of antioxidant substances and its peculiar acidic composition.

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