Effect of Olive Pulp Enrichment on Physicochemical and Antioxidant Properties of Wheat Bread

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Abstract

Black and green olive pulp was added to wheat bread formulation at different levels (5, 10, 15%) with the aim to improve its nutritional value by enhancing the phenolic content and antioxidant capacity. Additionally, the effects of the fortification with olive pulp on the physical characteristics, staling rate and overall consumer acceptability of the formulated breads were explored. Both olive pulps exhibited significantly higher antioxidant activity than refined wheat flour. Baking imparted an impressive increase in TPC, TFC and antioxidant activity of breads as revealed by comparison of experimental with theoretical values but returned significant differences only in the case of TPC when a two-tailed t-test for paired data was applied. Texture measurements showed a substantial increase in hardness with storage along with decreasing loaf volume and increased density. Hydroxytyrosol was the major phenolic compound of fortified breads followed by tyrosol. Olive pulp could be incorporated in a bread formulation without interfering with the general sensory acceptability.

Keywords: Olive pulp; Bread; HPLC; Antioxidants; Staling

1 Introduction

Traditional Mediterranean eating culture relies on ancient recipes composed of ingredients mainly derived from agricultural material from the rural area. The last few years have seen a tremendous back-to-nature demand in the market, probably because of an increased production of food with little nutritional value or even unhealthy food infected with some form of toxicity. The later has led to an increase in the use of natural antioxidants, especially those of vegetable origin like the case of table olives.

For several years now, the term "functional foods" has been used to define natural and naturally produced food with enrich characteristics that enhance consumer well-being. At this point, the demand for wheat-based products with value-added is growing rapidly (Bhattacharya, Langstaff, & Berzonsky, 2003). In the last decade, there is an increased tendency to produce functional breads made from whole grain

flour or other functional ingredients (Dewettinck et al., 2008). In the light of this, many ingredients or extracts have been included in bread formulations to increase their diversity, nutrition or product appeal. Among functional ingredients, antioxidants occupy a place of prominence since they may inhibit lipid peroxidation and improve bread quality and safety (Duan, Zhang, Li, & Wang, 2006). However, in the literature there are no studies concerning the addition of table olives in bread formulations. Table olives are a traditional component of the Mediterranean diet and their antioxidant properties and other biological activities are well studied. Part of the beneficial nutritional value of this diet has been attributed to olive oil owing to its high content of monounsaturated fatty acids and its minor constituents such as tocopherols and various phenolic components (Galli & Visioli, 1999). Although, olive products and by-products are known for their biological properties, there is a lack of research concerning the incorporation of pulp from processed green or black table olives in a bread formulation. The aim of this study was to improve the nutritional value of wheat bread with the incorporation of green or black olive pulps thus enhancing the phenolic content and antioxidant capacity. Additionally, the effect of the supplementation of green or black olive pulp on the physical characteristics and staling rate of the formulated breads was explored.

2 Materials and Methods

2.1 Materials

Wheat flour (70% of milling yield) was purchased from a local milling company. The samples of green (Spanish type, cv. Conservolea) and black (Greek type, cv. Kalamon) table olives were purchased from the local market and used throughout in the present study. Both types of olives were pulped in a laboratory homogenizer after removal of the cores.

Reagents and chemicals

Hydroxytyrosol (HTR), tyrosol (TYR), oleuropein (OLE), protocatechuic acid (PRCA), 4-hydroxybenzoic acid (4HBA), vanillic acid (VA), caffeic acid (CA), syringic acid (SRA), *p*-coumaric acid (pCA) and ferulic acid (FA) were supplied by Sigma-Aldrich (Steinheim, Germany). Galic acid (GA) and catechin (CAT) were obtained from Extrasynthese (Genay Cedex, France). Analytical grade supplies of Folin-Ciocalteu, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 6-hydroxyl-2,5,7,8-tetramethychromane-2-carboxylic acid (Trolox) and 2, 4, 6-tripyridyl-s-triazine (TPTZ) were from Sigma-Aldrich (Steinheim, Germany). All other solvents/chemicals obtained from Chem-Lab (Zedelgem, Belgium) were of analytical grade or high-performance liquid chromatography (HPLC) grade.

2.2 Methods

Proximate chemical analysis

Moisture, protein and ash content in flour and formulated breads, were determined according to the official methods AACC 44-15A, ICC 105/2 and 104/1, respectively (AACC, 2000; ICC, 1994). Moisture content of olive pulps was determined by drying at 70°C until constant weight, whereas their ash content was determined by incineration at 550±15°C (AOAC 955.04 method) (AOAC, 1990). Total fat content was determined in a Soxhlet apparatus according to AOAC 948.22 method (1990), using petroleum ether as solvent with a minimum extraction time of 24h. Protein content was determined by the Kjeldahl method after multiplying by a factor of 5.7 for wheat flour and 6.25 for olive pulps. The carbohydrate content was calculated by difference as the percent remaining after all the other components have been measured.

Farinograph and bread making procedure

The mixing properties of the doughs obtained from the different flour-olive pulp blends were examined with a Brabender farinograph (OHgG, Duisburg, Germany), equipped with a 300 g mixing bowl according to ICC standard method No 115/1 (ICC, 1994).

All bread formulations contained wheat flour

(300 g, 14% moisture basis), olive pulp (black or green), salt (2% flour basis), dried yeast (1.5% f.b.) and water as determined by the farinograph water absorption in order to obtain 500 BU consistency. Green and black olive pulps were added fresh (not dried) at 0, 5, 10 and 15% supplementation levels (on dry matter basis). The salt content of the green and black olives was 8.5 and 7.0%, respectively, according to the labeling of the product by the manufacturer. Thus, no salt was added to the formulations where the salt of the pulp exceeded 2% of salt on flour basis, required by the recipe. Each fortification level experiment was carried out in duplicate.

Bread doughs were prepared using a two-step bulk fermentation and proofing, up to optimum volume increase of 100 g dough. Loaf volume was determined by rapeseed displacement. Subsequently, breads were sealed in polyethylene bags to monitor changes in bread characteristics upon storage (5^oC). Freeze-dried samples of bread crumbs were ground and homogenized, and finally used for measuring phenolic compounds and antioxidant activity.

Texture profile analysis (TPA)

A two bite TPA test was performed to measure the hardness, cohesiveness and chewiness of bread crumbs, from slices of the center of each bread loaf using a texture analyzer TA-XTplus (Stable Micro Systems, Gudaiming, Surrey, UK), equipped with P/50 probe. TPA analysis was carried out according to the AACC Method 74-09 (2000). For each test, 40% strain at a distance of 1.0 cm was applied to 25 mm thick samples. The test speed was 2.0 mm/s. TPA and bread moisture measurements were performed after 1, 3 and 5 days of storage at 5^oC in order to monitor staling and moisture loss. Mean values of at least three bread loaves were utilized for statistical purposes.

Color measurement

The color of bread crumbs was measured one-day after baking with a Hunterlab colorimeter, model MiniScan XE plus. Color readings were expressed by CIElab (L*, a*, b* and ΔE^*). Total color difference ΔE was calculated as $\Delta E^*=[(\Delta L^*)2+(\Delta a^*)2+(\Delta b^*)2]1/2$. Reported values are the means of independent triplicate measurements; two values obtained from the same loaf were averaged into one replicate.

Image acquisition and analysis

Images of bread slices were scanned using a scanner (HP ScanJet 3400C, Hewlett Packard,California, USA) in order to perform image analysis as described by Skendi, Biliaderis, Papageorgiou, and Izydorczyk (2010) with an UTHSCSA ImageTool programme (Version 3.0).

Total phenolic (TPC), total flavonoid content (TFC) and antioxidant activity assays

Estimation of free phenolic and flavonoid contents as well as antioxidant activity were performed in 80% methanol extracts. A double extraction (ratio 1:10) in an ultrasonic bath for 10min at 60^oC was applied on each freeze-dried sample (bread crumb or olive pulp). The mixture was then centrifuged at 4000 rpm, at 4^oC for 10min and the prepared extracts were stored in the freezer until analysis. Extraction was carried out at least in triplicate.

The amount of TPC in extracts was determined according to the Folin-Ciocalteu method (Irakli, Samanidou, Katsantonis, Biliaderis, & Papadoyannis, 2016; Singleton, Orthofer, & Lamuela-Raventos, 1999). The results were expressed as mg of GA equivalents per 100 g of the dried sample (mg GAE/100 g dw). The concentration of TFC was determined using the aluminum chloride colorimetric method (Bao, Cai, Sun, Wang, & Corke, 2005; Irakli et al., 2016). The TFC was estimated using a standard calibration curve, with catechin as a standard, and expressed as milligrams catechin equivalent (CATE) per 100 g of dry weight (mg CATE/100 g dw). The DPPH free radical scavenging activity (RSA) was based on the protocol described by Yen and Chen (1995). Results were expressed as mg Trolox equivalents per 100 g of dry sample (mg TE/100 g dw). The ferric reducing antioxidant power assay (FRAP) was performed according Benzie and Strain (1996) and Irakli et al. (2016). The results were expressed as mg Trolox equivalents per 100

g of dry sample (mg TE/100 g dw).

The ABTS radical scavenging activity was evaluated by employing the ABTS assay (Re et al., 1999). The ABTS•+ scavenging capacity was expressed as previously described for the DPPH assay.

HPLC profile of phenolic compounds

The analyses were performed on an HPLC Agilent 1200 system (Agilent Technology, Urdorf, Switzerland) equipped with a 250 x 4.6 mm i.d., 5 μm Nucleosil 100 C_{18} column (MZ, Mainz, Germany) maintained at 30°C, a 20μL loop and diode-array detector (DAD). The mobile phase consisted of three solvents: (A) 1% acetic acid in water, (B) acetonitrile and (C) methanol and the following gradient program was performed: 0min, 90% A-0% B; 10min, 80% A-4% B; 25min, 75% A-5% B; 30min, 65% A-5% B; 31min, 40% A-0% B; 37min, 35% A-20% B; 50min, 20% A-80% B. The flow rate of mobile phase was 1.3mL/min. The DAD recorded the spectra at 260, 280 and 320 nm and the chromatograms were analyzed using the Agilent Chemstation software (version B.04.01, Agilent Technologies). Identification of phenolics was obtained by comparison of retention times and UV/VIS spectra with those of authentic standards (Cabrera-Banegil et al., 2017).

Sensory evaluation

Sensory evaluation was carried out on the breads on the same day of baking. Seven breads were evaluated (control, fortified with 5, 10 & 15% green or black olive pulp) by a 20-member trained panel using a nine-point hedonic scaling method (1-9 scoring): 1=extremely dislike (or lowest quality), 5=either like or dislike (or medium quality) and 9=extremely like (or highest quality), respectively. The breads were sliced into equally sized pieces (1cm thick) and served coded, randomized. Crust and crumb color, flavor/aroma and taste, as well as overall acceptability, were evaluated. Breads were considered acceptable if their mean score for overall acceptability was above 5 (neither like nor dislike).

Statistical analysis

All experiments, unless otherwise mentioned, were performed in triplicate. Data were analyzed by analysis of variance (ANOVA) using the Duncan's multiple range test to detect significant differences ($p<0.05$). Differences between the mean values of nutritionally important constituents measured experimentally in the breads and the theoretical values expected from the contribution of each individual raw material (flour and olive pulp) in the bread recipe were evaluated by the two-tailed t-test for paired data, in order to appreciate the impact of the baking process on these components. The statistical analyses were performed using SPSS statistical software (IBM SPSS Statistics version 19, 2010).

3 Results and Discussions

3.1 Proximate chemical composition of olive pulps and wheat flour

The proximate chemical composition, TPC, TFC and total antioxidant activity (ABTS, DPPH and FRAP assays) of black or green olive pulps and of wheat flour are presented in Table 1. The results showed that there were significant differences in the examined parameters among the samples (wheat flour, black olive, green olive). Both moisture and crude fat content were significantly different among the black and green olive pulp. Similar results were observed for the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity assays (ABTS, FRAP and DPPH). The differences observed are probably related to factors such as cultivation practices, ripeness, harvesting time, climatic conditions and storage time of table olives (Pereira et al., 2006). In particular, due to the different ripening stage, the fat content of the green olives was 45% less than of the black, while crude protein, ash and polysaccharide contents were similar in both pulps. Black olive pulp had a richer TPC when compared to the green and both accounted for 5 and 3 times the TPC of wheat flour, respectively. The TFC of the black olive pulp was more than 1.4 times higher than that of the green

Table 1: Proximate chemical composition, total phenolic content, total flavonoid content and antioxidant activity (ABTS, FRAP, DPPH) of wheat flour, green and black olive pulp

Parameters (%)	Wheat flour	Black olive pulp	Green olive pulp
Moisture content	13.29 (±0.12)[1]	60.24 (±0.55)[2]	75.08 (±0.07)[3]
Crude protein	13.11 (±0.23)[2]	2.89 (±0.13)[1]	2.85 (±0.10)[1]
Crude fat	1.30 (±0.03)[1]	32.11 (±0.06)[3]	17.65 (±0.38)[2]
Ash content	0.58 (±0.01)[1]	1.61 (±0.01)[2]	1.50 (±0.21)[2]
Polysaccharide content (by difference)	71.72	3.45	2.92
TPC (mg GAE/100g)	70.23 (±0.32)[1]	344.75 (±28.64)[3]	246.10 (±17.11)[2]
TFC (mg CAT/100g)	52.92 (±0.02)[1]	320.60 (±30.69)[3]	219.90 (±24.89)[2]
ABTS (mg TE/100g)	25.61 (±2.62)[1]	434.90 (±28.14)[3]	288.20 (±18.95)[2]
FRAP (mg TE/100g)	24.35 (±0.22)[1]	476.75 (±28.78)[3]	302.40 (±16.40)[2]
DPPH (mg TE/100g)	20.60 (±0.85)[1]	442.45 (±16.67)[3]	272.10 (±15.41)[2]

*Reported values are the mean of at least 3 replicates.
**Different numbers in the same line used as superscripts indicate differences ($p<0.05$) amongst the means, as determined by the Duncan's multiple range test.

olive pulp but they are both richer in flavonoids when compared to refined wheat flour. Antioxidant activity measurements from ABTS, DPPH and FRAP assays show that again the black olive pulp exhibited higher antioxidant activity than its green counterpart. Both olive pulps exhibit significantly higher antioxidant activity than refined wheat flour and can serve their role as nutritional enhancers in a bread recipe.

3.2 Mixing characteristics of dough

Fortification with olive pulp resulted in lower water absorption and longer development time, and produced more stable dough with a lower degree of softening than the control (data not shown). The decreasing water absorption on the farinograph follows the pattern of increased intrinsic moisture content of the olive pastes (Table 1) and the increase in supplementation level. More than 5% inclusion of olive pulp in wheat dough increased the dough development time (DDT) by 0.6-1.5min, in the faringraph, as well as the mixing stability from 4.3 to 7.0min. There is an obvious delay in the development of the gluten network in the presence of olive pulp. However, the dough remained consistent for a longer time

when supplemented with olive pulp as evidenced by longer stability times and by lower values for degree of softening.

3.3 Physicochemical characteristics of bread

The characteristics of breads prepared after fortification with green or black olive pulp are summarized in Table 2. As it can be seen there is a significant progressive decrease in moisture content of all loaves on storage. However, the formulated breads with black olive pulp at higher levels of pulp addition managed to retain their moisture for longer. The higher moisture content of breads containing olive pulp may be related to the presence of fat which prevents water evaporation during the baking process (Pareyt, Finnie, Putseys, & Delcour, 2011). The weight loss of all breads was ~10%, while there was a pronounced progressive decrease of loaf volume with increasing addition of olive pulp. This may be attributed to the dilution of the gluten network and the resulting decrease of gas retention capacity. Thus, olive pulp although a high in fat ingredient does not act as a typical shortening which is expected to entrap air during mixing thus enhancing the loaf volume. In the case of

Table 2: Moisture content on storage, weight loss, loaf volume, crumb color and gas characteristics of control bread and of formulated breads with the addition of olive pulp at different supplementation levels (5, 10, 15%)*

	Control	Black olive pulp			Green olive pulp		
		5 %	10%	15%	5 %	10%	15%
Moisture content (%)**							
Day 1	40.06 (±0.3)[1,2,a]	42.15 (±0.4)[3,4,a]	42.60 (±0.4)[3,4,a]	43.70 (±0.2)[4,a]	40.25 (±0.1)[1,2,a]	41.47(±0.6)[2,3,a]	39.69(±0.1)[1,a]
Day 3	38.05 (±0.1)[1,b]	39.80 (±0.1)[2,b]	40.23 (±0.3)[2,a,b]	41.85 (±0.3)[3,a,b]	37.56 (±0.2)[1,b]	40.48(±0.2)[2,a,b]	37.60(±0.1)[1,b]
Day 5	34.34 (±0.1)[1,c]	38.11 (±0.4)[2,c]	38.16 (±0.6)[2,b]	39.93 (±0.5)[2,b]	34.56 (±0.2)[1,c]	38.19(±0.4)[2,b]	33.18(±0.1)[1,c]
Weight Loss (%)[a]	10.0	9.3	10.0	10.1	9.9	10.1	10.6
Loaf volume**							
Volume (cm^3)	235 (±10.0)[1]	225 (±2.8)[1,2]	195 (±10.6)[13]	160 (±15.2)[4,5]	215 (±21.2)[2]	170 (±3.5)[4]	150 (±5.0)[5]
Decrease (%)[b]	-	4.30	17.02	31.91	8.50	27.66	36.17
Crumb color**							
L*	67.22 (±0.62)[1]	62.95 (±0.30)[2]	62.48 (±0.13)[2]	59.45 (±0.96)[3]	48.02 (±0.53)[4]	44.23 (±0.57)[5]	38.12 (±0.34)[6]
a*	1.70 (±0.28)[5]	2.51 (±0.23)[3,4]	3.62 (±0.02)[2]	4.75 (±0.06)[1]	2.27 (±0.12)[4]	2.50 (±0.23)[3,4]	2.61 (±0.31)[3]
b*	15.85 (±0.23)[3]	9.56 (±0.17)[4]	9.34 (±0.09)[4,5]	9.13 (±0.16)[5]	17.16 (±0.04)[2]	17.37 (±0.28)[2]	20.46 (±0.18)[1]
ΔE*	-	20.22	23.97	30.02	4.50	5.04	9.08
Gas characteristics							
Total cells	632 (±58)[1]	366 (±25)[3]	365 (±19)[3]	397 (±21)[4]	328 (±70)[4]	338 (±60)[3,4]	348 (±38)[3,4]
Nr of cells ≤4mm^2	371(±23)[1]	240(±38)[3]	214(±29)[3,4]	222(±33)[3,4]	189(±41)[4]	190(±35)[4]	224(±31)[3,4]
Total cell area (mm^2)	101.1(±14)[1]	49.5(±23)[2,3]	68.2(±16)[2]	37.3(±28)[3]	57.4(±24)[2,3]	51.7(±17)[2,3]	35.6(±32)[3]
Mean cell area (mm^2)	0.16(±0.09)[1,2]	0.14(±0.10)[1,2]	0.19(±0.07)[1]	0.09(±0.06)[2]	0.17(±0.08)[1,2]	0.10(±0.09)[1,2]	0.10(±0.09)[1,2]
Nr cells/cm^2	70.2(±3.28)[1]	40.7(±1.12)[3]	40.5(±1.03)[3]	44.2(±1.02)[2]	36.4(±2.42)[4]	37.5(±2.12)[3,4]	38.6(±1.01)[4]

*Reported values are the mean of at least 3 replicates.
**Different numbers in the same line used as superscripts indicate differences (p<0.05) amongst the means, as determined by the Duncan's multiple range test.
[a] Weight loss calculation based on 100 g dough used for each loaf of bread
[b] Compared with the control

olive oil $\sim75\%$ percent of its fat content is oleic acid (Bartoli et al., 2000). The highest value of L^* corresponding to lightness was obtained for the control bread. Addition of olive pulp significantly decreased L^* proportionally to the level of supplementation and the type of olive pulp. In the case of a^*, the addition of olive pulp signified predominance of red over green in the bread crumb. In all cases, the value of b^* was positive, which corresponds to a more intense yellow hue over blue both in control and fortified breads. Introduction of black or green olive pulp even at the lowest supplementation level resulted in a dramatic decrease of ≈42 and $\approx48\%$ of total cells, respectively. It seems that the air bubbles entrapped in oil cannot withstand baking or even proofing as opposed to those incorporated in the gluten matrix. The observed decrease in total cell area was confirmed by the decrease in volume yield of breads containing olive pulp as well as by the visual detection of higher density breads. A significant decrease was also observed in the number of cells $\leq4mm^2$, and number of cells per cm^2 with introduction of olive pulp. During mixing, the incorporated air forms gas cells as nucleation sites for the CO_2 gas generated by yeast activity during proofing. The three-dimensional protein network traps gasses and the embedded gasses expand the dough, leading to a porous structure after baking (Campbell, 2003). In the case of breads with olive paste the significant fat content of both black and green olives also incorporate air on mixing. As was previously reported (Jacob & Leelavathi, 2007), the air incorporated into oil cannot be retained thus leading to an increase in hardness of bread. The later is in agreement with our findings in the following section.

3.4 Texture profile analysis of wheat flour bread and of breads containing olive pulp

Fig. 1 shows the texture parameters of the control bread and of breads containing black or green olive pulp. Hardness of breads containing black olive pulp (Fig. 1a$_1$) did not differ from the control as a function of increased supplementation level up to 10% olive pulp during the whole stor-

age period. In the case of breads with green olive pulp only the bread with 5% green olive pulp had similar hardness values with the control during the whole storage period. Generally, there was a substantial increase in hardness with storage time for breads at 10 and 15% supplementation (Fig. 1a$_2$). The increase in bread hardness may result from a decrease in the total area of the gas cell; greatest crumb hardness is usually observed in breads with the lowest loaf volume. Besides, hardness values of breads with the addition of 5, 10 or 15% green olive pulp (Fig. 1a$_2$) were higher than the corresponding breads with black olive pulp (Fig. 1a$_1$). The higher fat content of the black pulp could act as a moisture barrier or can be engaged in retardation of starch retrogradation (Provost, Colabroy, S. Kelly, & Wallert, 2016). Results of cohesiveness (Fig. 1b$_1$, b$_2$) indicated that for all breads, cohesiveness followed a decreasing trend on storage and increased supplementation with olive pulp. This is due to the crumb offering less resistance to the compression force. The observed decrease of cohesiveness on storage could be attributed to moisture loss of the breads (Table 2). Besides, the decreased cohesiveness may be related to the loss of intramolecular attraction among ingredients as postulated by Gomez, Ronda, Caballero, Blanco, and Rosell (2007) thus resulting in increased susceptibility of the bread to crumbliness (Boz & Karaoglu, 2013). As far as the chewiness of breads is concerned (Fig. 1c$_1$, c$_2$), a similar pattern to hardness was observed.

3.5 Antioxidant properties of breads

The TPC of the control and fortified breads with black or green olive pulp is shown in Fig. 2. The highest TPC values were observed in breads fortified with 15% olive pulp. The TFC of the breads containing black olive pulp was higher than those with green olive pulp. Besides, the results indicated that as the supplementation level increases the ABTS values of fortified breads increased significantly ($p<0.05$). As regards the values of the DPPH assay for the fortified breads with black and green olive pulp they ranged between 20.7 to 65.1 mg TE/100 g and 11.9 to 40.5 mg TE/100
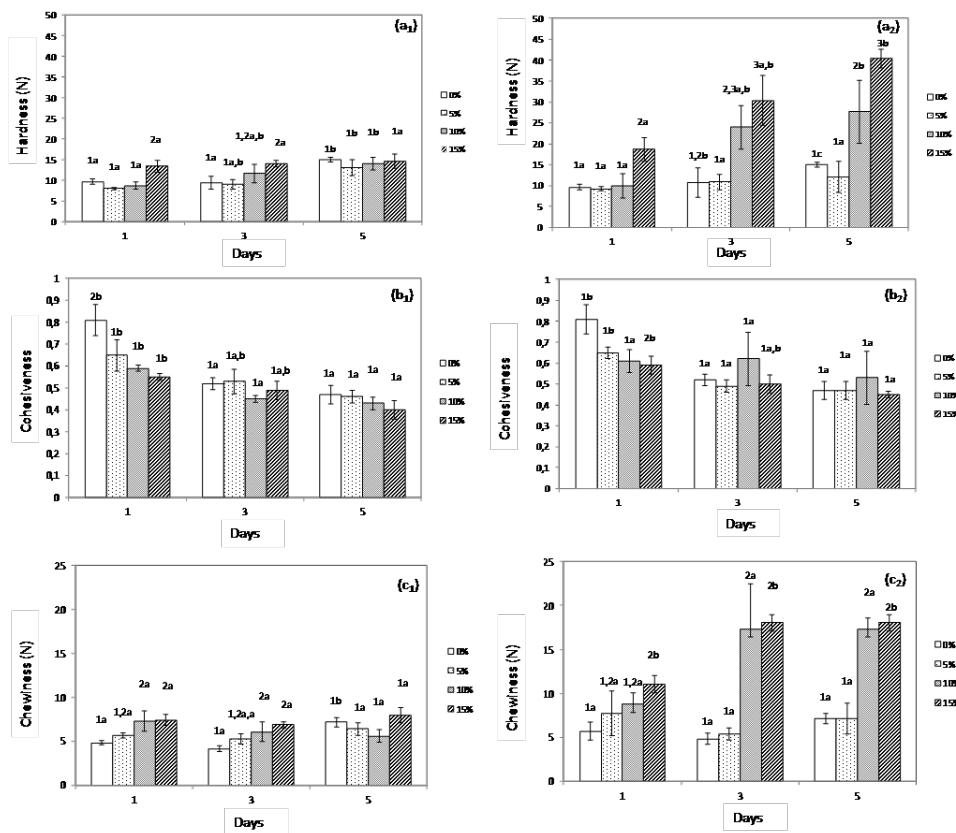
Figure 1: Texture profile parameters: Hardness (a_1, a_2); Cohesiveness (b_1, b_2); Chewiness (c_1, c_2) of control bread (0%) and of breads containing black (a_1, b_1, c_1) or green (a_2, b_2, c_2) olive pulp. Different numbers within the same day group or letters for the same recipe (on different days) used as superscripts indicate differences ($p<0.05$) amongst the means, as determined by the Duncan's multiple range test.

g, respectively, whereas the control bread showed 6.46 mg TE/100 g DPPH radical scavenging activity. This was expected since Maillard reaction products also possess certain free radical scavenging activity (Jing & Kitts, 2000). The same trend was observed for the FRAP assay. As for the effect of storage time, according to Fig. 2, there is no significant difference of antioxidants, irrespective of the assay employed during the whole period of storage.

Many researchers, observed an increase of the TPC and the antioxidant activity of the baked product (bread) compared to raw flour (Gelinas & McKinnon, 2006; Holtekjolen, Baevre, Rodbotten, Berg, & Knutsen, 2008; Yu, Nanguet, & Beta, 2013). In an attempt to investigate the

role of the baking process, Table 3 shows the experimental and the theoretical values (by calculation) of TPC and antioxidant activity (ABTS, FRAP, DPPH) of the control bread and of fortified breads, on the first day of storage. Instead of comparing the antioxidants of raw materials (wheat flour and olive pulps) to those of the final product (bread) we proceeded to the calculation of theoretically expected values, based on the actual contribution of dry ingredients per 100 g of dry bread while taking into account weight loss from dough to bread (Table 1). Furthermore, we employed the paired sample t test to reveal if mean differences between the experimental and theoretical values were significant ($p<0.05$). This test was employed in two groups; group 1
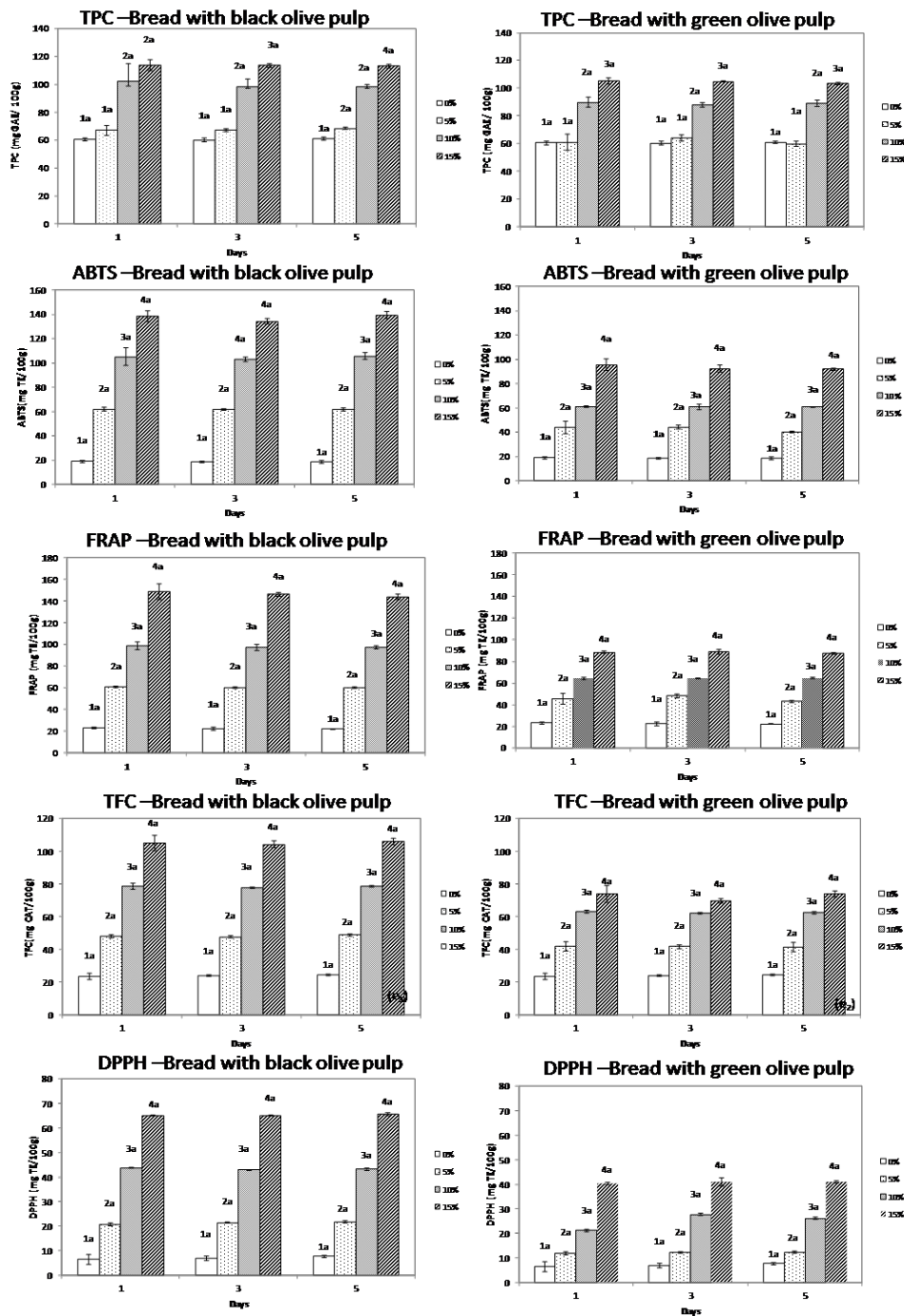
Figure 2: Total phenolic content, total flavonoid content and antioxidant activity (ABTS, FRAP, DPPH) of control bread and of breads containing black or green olive pulp at 5, 10 or 15% supplementation levels. Different numbers in the same day group or letters among the same recipe (on different days) used as superscripts indicate differences ($p<0.05$) amongst the means, as determined by the Duncan's multiple range test.
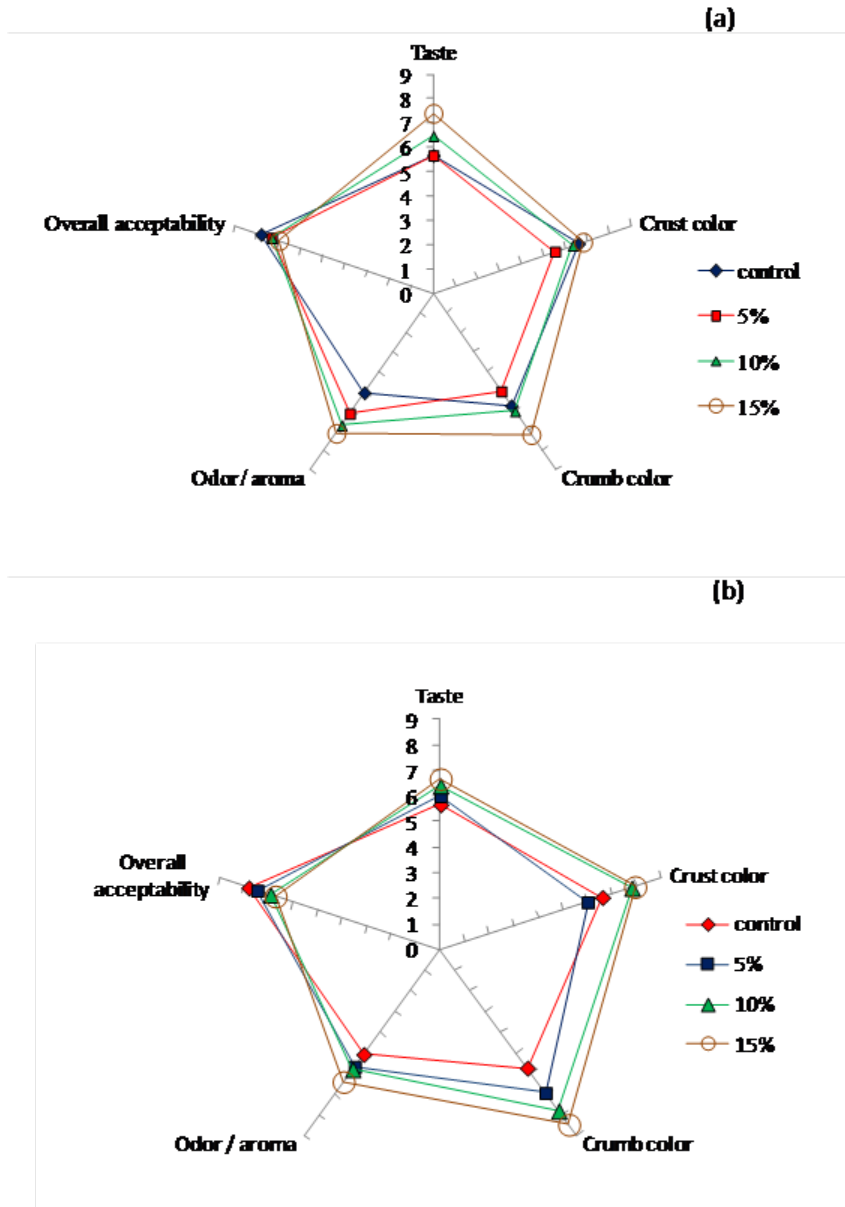
Figure 3: Sensory evaluation of black (a) and green (b) olive pulp supplemented breads

Table 3: Experimental mean value and theoretical values (by calculation) of TPC, TFC and antioxidant activity (ABTS, FRAP, DPPH) of control bread and of breads containing black or green olive pulp at supplementation levels 5, 10, 15% on storage day 1. Values in brackets show standard deviation

| | Control | Formulated breads with black olive pulp | | | Formulated breads with green olive pulp | | |
		5%	10%	15%	5%	10%	15%
TPC (mg GAE/100 g)			Group 1*			Group 2*	
Experimental	60.5 (±1.3)	67.0 (±3.5)	102.2 (±13.0)	114.1 (±3.9)	60.9 (±5.8)	89.7 (±3.6)	105.2 (±2.2)
Theoretical	24.2	30.1	35.3	40.3	29.1	32.5	38.5
TFC (mg CAT/100 g)			Group 1			Group 2	
Experimental	23.6 (±2.0)	48.1 (±1.0)	78.6 (±1.7)	105.1 (±4.6)	41.9 (±2.0)	62.9 (±2.9)	73.8 (±1.0)
Theoretical	18.3	23.4	28.0	32.3	18.3	24.7	29.7
FRAP (mg TE/100 g)			Group 1			Group 2*	
Experimental	22.9 (±0.8)	60.9 (±0.8)	98.8 (±3.7)	148.7 (±7.0)	45.5 (±4.9)	64.4 (±0.9)	88.4 (±0.9)
Theoretical	8.4	17.3	25.5	33.1	14.5	19.6	26.3
ABTS (mg TE/100 g)			Group 1			Group 2	
Experimental	18.8 (±1.2)	61.9 (±1.5)	105.2 (±7.3)	138.5 (±14.4)	43.7 (±5.0)	61.1 (±0.9)	95.5 (±4.7)
Theoretical	8.8	16	24.3	31.3	14.6	19.4	25.7
DPPH (mg TE/100 g)			Group 1			Group 2	
Experimental	6.5 (±2.0)	20.7 (±0.5)	43.7 (±0.2)	65.1 (±0.1)	11.9 (±0.6)	21.2 (±0.4)	40.5 (±0.5)
Theoretical	16.4	23.8	30.4	36.7	21.8	26.0	32.3

* Significant mean differences between the experimental and theoretical values if $p < 0.05$.

comprised the control and the formulated breads with black olive pulp and group 2 comprised the control and the formulated breads with green olive pulp.

The TPC of fortified breads with black olive pulp at 5% supplementation level was more than 2 times higher than that obtained by calculation (theoretical), while those at 10 or 15% supplementation level were 3 times higher than the theoretical one. In the formulated breads with green olive pulp at 5, 10 or 15% supplementation levels the experimental TPC was ~2.5 times higher than those of the theoretical one. Similarly, the TFC of the breads with the addition of black olive pulp at 5, 10 or 15% level were 2.0, 2.8 or 3.2 times higher compared to the theoretical ones, while those with green olive pulp were 1.7, 2.1 or 2.0 times higher, respectively. The formulated breads with black green olive pulp at 15% supplementation level had an antioxidant capacity (ABTS, FRAP) ~4.4 times more than the theoretical and with green olive pulp ~3.7 times. To the contrary DPPH experimental values of formulated breads with black or green olive pulp were only 1.7 or 1.2 times higher than that of the theoretical. As for the control bread, the experimental values of TFC, TPC, ABTS & FRAP were also higher in comparison with the theoretical. Despite the impressive increase that baking

imparted in all the examined parameters for all breads, the paired sample t test showed that the mean differences between the experimental and theoretical values (i.e. effect of baking) were only significant for the TPC of Groups 1 and 2 breads and for FRAP values of Group 2 breads. The observed differences between the experimental and the theoretical values of the control bread and of the formulated breads are attributed to the bread making process. In the literature, studies on the effect of baking on the TPC, TFC and antioxidants activity of bread are contradictory (Gelinas & McKinnon, 2006; Holtekjolen et al., 2008; Yu et al., 2013). The antioxidant composition or capacity of bakery products derives from the intrinsic phenolic compounds of flour, added phenolic ingredients, other ingredients physically containing phenolics, intermediate phenolic products newly generated during baking for example, via Maillard reactions (Michalska, Amigo-Benavent, Zielinski, & del Castillo, 2008), thermal-induced degradative products (Rupasinghe, Wang, Huber, & Pitts, 2008), and/or polyphenol polysaccharides complexes (Shahidi & Naczk, 1995). In accordance with our results, Chandrasekara and Shahidi (2011) reported that products of Maillard reactions could increase the TPC of baked breads when compared to raw flour. However, it cannot be specified whether the observed dif-

Table 4: Content (μg/g) of phenolic compounds on dry-weight basis of flour, black or green olive pulps, and of control and formulated breads with black or green olive pulp at supplementation levels of 5, 10, 15%

Analyte	Flour	Black olive pulp	Green olive pulp	Control (Bread)	Breads with Black olive pulp			Breads with Green olive pulp		
				0%	5%	10%	15%	5%	10%	15%
Phenolic acids & their derivatives										
HTR	ND	788.6(±17.1)	321.9(±17.1)	ND	135.2(±12.1)	278.6(±10.3)	481.4(±20.5)	57.0(±6.3)	77.0(±4.1)	98.4(±8.8)
PRCA	0.2(±0.0)	2.7(±0.1)	1.6(±0.2)	0.2(±0.0)	0.7(±0.1)	1.0(±0.2)	1.4(±0.3)	0.8(±0.1)	1.0(±0.1)	0.7(±0.1)
TR	ND	280.6(±34.6)	49.1(±5.7)	ND	25.2(±2.5)	74.1(±6.3)	124.2(±10.1)	23.6(±1.8)	28.2(±6.5)	32.8(±5.6)
4HBA	0.5(±0.1)	2.7(±0.2)	1.7(±0.5)	0.5(±0.1)	0.8(±0.1)	2.0(±0.2)	2.1(±0.3)	1.0(±0.2)	0.8(±0.1)	0.7(±0.2)
VA	0.9(±0.2)	12.8(±2.8)	2.8(±0.6)	0.9(±0.2)	2.5(±0.4)	6.2(±0.8)	8.5(±0.5)	2.1(±0.3)	2.3(±0.2)	2.4(±0.1)
CA	ND	2.7(±0.1)	2.7(±0.4)	ND	0.8(±0.1)	1.0(±0.2)	1.0(±0.3)	0.5(±0.1)	0.4(±0.0)	0.3(±0.0)
SRA	0.1(±0.1)	2.0(±0.1)	0.5(±0.1)	1.0(±0.3)	0.6(±0.1)	0.6(±0.1)	0.7(±0.2)	0.2(±0.0)	0.6(±0.2)	0.2(±0.0)
PCA	0.3(±0.0)	ND	5.7(±0.1)	0.3(±0.0)	1.1(±0.3)	1.4(±0.4)	1.3(±0.2)	0.8(±0.1)	0.6(±0.1)	0.4(±0.1)
FA	0.5(±0.1)	ND	ND	0.5(±0.1)	0.5(±0.1)	0.6(±0.1)	0.6(±0.1)	0.6(±0.1)	0.6(±0.2)	0.4(±0.1)
OLE	ND	105.6(±0.4)	ND	ND	ND	ND	ND	ND	ND	ND
Total μg/g	2.0(±0.4)	1197.7(±60.7)	385.8(±55.6)	3.4(±0.1)	167.2(±15.5)	364.8(±18.5)	620.7(±33.3)	86.0(±9.1)	110.7(±11.6)	135.8(4.8)

ND-Not detected.
Values are means of duplicate analysis.

ferences in the TPC could be attributed to the fermentation, kneading or the baking process.

3.6 HPLC profile of phenolic compounds

Table 4 shows the content of phenolic acids and their derivatives for wheat flour, black or green olive pulp and formulated breads. The black olive pulp contained higher quantities of phenolic compounds compared to its green counterpart. Hydroxytyrosol was the major phenolic compound existing in both black and green olives, with the black olive pulp exhibiting the highest concentration. This was expected since hydroxytyrosol is considered the major phenolic compound existing in processed olives followed by tyrosol (Boskou, 2017). OLE was detected only in black olives with a mean value of 105.6 μg/g. The detectable content of the rest phenolic compounds (sum of PRCA, 4HBA, VA, CA, SRA and PCA) ranged between 15.0 and 22.9 μg/g for green and black olive, respectively, whereas FA was not detected in both types of olives. The formulated breads with 5 to 15% black or green olive pulp presented approximately 50 to 200 times or 25 to 40 times greater amount of phenolic compounds compared to the control. In particular, the HPLC analysis showed that HTR was the major phenolic compound in formulated breads with 5 to 15% black or green olive pulp ranging from 135.2 to 481.4 μg/g and 57 to 98.3 μg/g, respectively. A similar trend was observed for the TR, the second primary phenolic compound in formulated breads with black and green olive pulp, ranging from 25.2 to 124.2 μg/g and 23.6 to 32.8 μg/g, respectively as the fortification level increased from 5 to 15%.

3.7 Sensory evaluation of wheat flour bread and of breads containing olive pulp

The sensory evaluation scores of breads with different supplementation levels of green or black olive pulp is shown in Fig. 3. The highest taste scores were obtained in the formulation with 15% black olive pulp. Crust and crumb color as well as the taste of breads containing olive pulp scored higher than the control. The remarkable color difference measured instrumentally did not seem to affect the scoring of the breads for crust or crumb color. In general, the rating in taste, crust and crumb color, and odor/aroma increased with the supplementation level. Breads at 10 and 15% supplementation level of black olive pulp scored significantly higher. All formulations with olive pulp were acceptable, since they received scores for overall acceptability, much higher than 5, ranging from 6.73 to 7.47. The results suggest that supplementation of olive pulp in a wheat bread formulation would not interfere with general bread acceptability.

4 Conclusion

Fortification of wheat bread with preferably black rather than green olive pulp resulted in tasty breads with satisfactory overall acceptability despite the denser structure and smaller loaf volumes observed. The formulated breads with black olive pulp at higher levels of pulp addition managed to retain their moisture for longer, possibly due to the higher fat content to its green olive counterpart, and also exhibited a slower staling rate. As shown by TFC, TPC, FRAP, ABTS and DPPH assays, it can be concluded that the formulated breads with olive pulp, had significantly higher antioxidant activity as compared to the control whereas the formulated breads with black olive pulp had higher antioxidant activity than the green-olive counterparts. The observed antioxidant activity remained unchanged during the 5 days of storage, for all bread formulations with olive pulp. HPLC results showed that HTR was the major phenolic compound in formulated breads, followed by TR.

Acknowledgements

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