Egyptian Journal of Food Science
http://ejfs.journals.ekb.eg/

Quality Evaluation, Nutrition Values, and Shelf-life Stability of Bakery Products Supplemented with Dried Olive Mill Wastewater

Rokaya H. Jeba[1*], Hanaa M. Hemada[1], Abdel Aziz Nadi[2] and Mohamed E. Mansour[2]

[1]*Nutrition and Food Science Department, Faculty of Home Economics, Helwan University, Cairo, Egypt.*

[2] *Food Technology Department, National Research Centre, Dokki, Giza, Egypt.*

Olive mill wastewater (OMWW) is a by-product generated in large quantities during olive oil production. This study explored the benefits of incorporating dried OMWW into wheat dough and bakery products such as wheat bread and biscuits. The study investigated the effect of dried OMWW as a source of bioactive compound on dough rheological properties and properties of selected bakery products (quality, nutritional value, and shelf-life). It was detected that dried OMWW has a high concentration of total phenol (TP) 5.75 g GAE/L especially the oleuropein bioactive compound 235›2 mg/100g. The addition of OMWW (up to 3%) led to a decrease in water absorption and dough development time, while increasing dough softness and elasticity compared to the control. Bread volume increased with 3% OMWW supplementation. Scores for color, appearance, and overall acceptability of both bread and biscuits (without addition of lemon) decreased significantly ($p < 0.05$) compared to the control. The tenderness of OMWW-supplemented products improved significantly ($p < 0.05$) over 7 days of storage at 25°C. Oxidative stability of biscuits also improved with 3% OMWW supplementation after 3 weeks at 25°C, as evidenced by a significant ($p < 0.05$) reduction in thiobarbituric acid (TBA) values, though TBHQ (200 ppm) supplemented controls had the lowest TBA values overall. Additionally, OMWW supplementation increased the total phenol content and nutritional value of the bakery products. The study suggests that food industries should consider using dried OMWW in bakery products to create functional foods with enhanced nutritional profiles and extended shelf lives.

Keywords: OMWW, Rheological, Bakery, Supplementation, Shelf life, Nutritional value.

Introduction

The use of food additives is regulated by specific European Union EU laws, considering the food where it can be applied, maximum usable quantities, chemical characterization, and purity (Silva and Lidon, 2016[a]). According to the technological function, twenty-five families of food additives have been defined. Since the oxidation of foodstuffs is an important way of degradation, one of the major families of food additives is constituted by antioxidant agents (Silva and Lidon, 2016[b]). The use of synthetics antioxidant food additives is widespread in the agrofood industry in a wide variety of food stuffs. Some antioxidants have no danger at the dosages used as food additives, but the use of other antioxidants might trigger some side effects on consumers health. Natural antioxidant molecules are safer than synthetic antioxidants, available in complex forms, which include tocopherols, lycopenes, flavonoids, polyphenols,

[*]Corresponding Author: hossam@heco.helwan.edu.eg ; Tel: +201019119529
Received :8/12/2024; Accepted :6/2/2025
DOI: 10.21608/EJFS.2025.342784.1202

nordihydroguaretic acid (NDGA), sesamol, gossypol, vitamins, provitamins, and other phytochemicals, enzymes (catalase, glutathione peroxidase, super oxide dismutase), minerals (Zinc, Selenium), and lecithin (Lalani et al., 2024).

In recent years, due to increasing public awareness regarding health and wellbeing and apprehensions about using synthetic antioxidants in the food system, there has been a growing interest to identify and utilize antioxidative properties in many natural sources (Difonzo et al., 2021).The virgin olive-oil chain leads to the production of by-products (olive pomace and wastewater) and wastes (olive leaves and wood), which represent an important environmental issue in the Mediterranean areas, above all because they are generated in large quantities and in short periods of time (Banias et al., 2017). Natural phenols from olives and their by-products are now recognized as potential targets for the food, cosmetic, and pharmaceutical industries. Nowadays, interest in novel sources of natural antioxidants is steadily growing. Since OMWW is available in huge quantities and exhibits high concentrations of phenolic compounds, they may turn into a natural source of valuable and powerful antioxidants (Otero et al., 2021).

OMWW appears as a dark liquid; the water content represents between 70 and 90% of the total weight of OMWW; and it usually has a pH between 3 and 6 (slightly acidic) with a strong smell of olive oil (Flamminii et al., 2021). Phenolic chemicals found in OMWW include verbascoside, oleuropein, caffeic acid, ferulic acid, hydroxytyrosol, tyrosol, and their isomers/ derivatives (Azzam and Hazaimeh, 2021).

Bakery goods are some of the most consumed products in several cultures around the world. Bakery products are widely consumed in Egypt and represent the basis of daily nutrition for people of all ages. The basic ingredients are wheat flour and water. Regarding baking technology, the main differences lie in the leavening conditions, leavening agents, kneading methods, cooking temperatures, and moisture of the final product. Bakery products generally have a poor supply of phenolic compounds, especially those made with refined flour. The most common chemical spoilage occurs in bakery products is lipid oxidation which occurs especially in bakery products prepared with oil or fat (Arranz-Otaegui et al., 2018).

Bakery products are subject to chemical, physical, and microbiological spoilage. Spoilage refers to any change in the condition of a food that makes it less palatable at the time of consumption. The spoilage problems of bakery products can be sub-divided into: (a) physical spoilage (moisture loss, staling), (b) chemical spoilage (rancidity), and (c) microbiological spoilage (yeast, mold, bacterial growth) (Smith et al., 2004). However, incorporating phenolic compounds into cereal-based products, particularly baked goods, remains a persistent challenge for the food industry because of the low thermostability and aptitude to interact with nutritional components and compounds formed during the baking process, affecting the final color, texture, and flavor of products (Ou et al., 2019). Investigators (Subiria-Cueto et al., 2021) reported that using flours enriched with by-products which contained high phenolic compound levels increased the antioxidant capacity and fiber content; on the other hand, it decreased the overall sensory appeal.

Product shelf-life management is a crucial aspect of the baking industry, as baked goods have a limited shelf life and can easily spoil. This is one of the common challenges that baking businesses face (Smith et al., 2004). Therefore, it would be highly valuable to investigate the potential use of dried OMWW (a by-product of the olive industry) , as a source of bioactive compounds that may serve as natural antioxidants, and its effect on the rheological properties of wheat dough and the properties of bakery products (quality, nutritional value, and storage stability).

Materials and Methods

Materials

Olive fruits (*Olea europaea L.*) were used from the Koroneiki cultivar, the crop season of mid-November 2022, which was obtained from the Menoufia Governorate, Egypt. Butylated hydroxyl toluene (BHT), DPPH (2,2-Diphenyl-1-picrylhydrazyl), bromocresol green, sodium nitrite, tocopherol, gallic acid, folin–ciocalteu reagent, trichloroacetic acid (TCA), and other chemicals were obtained from Sky chemical co. Egypt. Wheat flour (72%), Whole wheat flour, sunflower oil, egg, sugar, lemon, salt, dry yeast, and baking powder for wheat pan bread and biscuit preparation were purchased from a local Egyptian market as reported in (Tables 1 and 2).

Methods

Extraction of olive mill wastewater

In the present study, olive oil and its by-products (olive mill wastewater and pomace) were produced using a machine that operates on a three-phase system. The olive fruits underwent several steps: washing with cold water, crushing, malaxation at 34ºC, and separation by centrifugation (KAIDA, TG18G, China). The obtained OMWW was dried according to Benincasa et al. (2022) with some modifications as following: OMWW was placed on a Petri dish in an oven (Zada, 2000 wt. Egypt) at 40 ºC until dried, then packaged in a plastic bottle and stored in freezer until use.

Polyphenol profile of dried OMWW by HPLC

The dried OMWW subjected to HPLC analysis according to the method reported by Cuffaro et al. (2024). 20 mL of 2 M NaOH was added to a 1 g sample in a quick-fit conical flask. Once flushing the flask with nitrogen gas, the stopper was sealed. At room temperature, the mixture was shaken for four hours. After that, 6M HCl was used to bring the pH down to 2. After centrifuging the samples for ten minutes at 5000 rpm, the supernatant was gathered. Two extractions were carried out using a 1:1 mixture of 50 mL of ethyl ether and ethyl acetate to extract the phenolic components. Following the separation and evaporation of the organic layer at 45 °C, the residues were r dissolved in two mL of methanol. HPLC analysis was carried out using an Agilent Technologies 1100 series liquid chromatograph equipped with an auto-sampler and a diode-array detector (Agilent Technologies, California, USA).

Determination of total phenol content (TP) and total flavonoids (TF)

Total phenol content was determined in dried OMWW, bread, and biscuits using the Folin–Ciocalteu reagent, according to Abdel-Moemin and Aborya (2014). The Folin–Ciocalteu method, as modified by Salazar-Lopez et al. (2016), was used to determine total phenolic content. Gallic acid (0–200 mg/mL) was used as a standard to derive the calibration curve (x=y-0.043/0.0088). Total phenolic content was expressed as mg of gallic acid (GA) per L of OMWW. The test is based on all phenolic compounds contained in the samples being oxidized by the Folin–Ciocalteu reagent. Total flavonoid content of OMWW was determined according to Valenzuela-González et al. (2022). The determination of total flavonoids in the OMWW was carried out in triplicate, and the results were averaged.

Preparation of supplemented bakery products with dried OMWW

The supplementation of bakery products (pan bread and biscuits) were done by partially replacing wheat flour with different percentages of dried OMWW 1% and 3% (2.6, 7.8g; respectively), as shown in (Tables 1and 2), with the aim to produce functional bakery products. The preparation of pan bread and biscuit were done according to the methodology reported by Chaple et al. (2023). The standard formula for products supplementation was presented in (Tables 1 and 2).

Rheological properties determination of supplemented wheat flour

Rheological properties of wheat flour (72%) supplemented with dried OMWW for products preparation (with lemon and without lemon) were investigated compared with the respective attributes of the control $^{(-)}$ wheat flour without and with supplementation with TBHQ (200 ppm). Rheological investigations were determined by farinograph and extensograph tests to examine the effect of dried OMWW supplementation on dough gluten formation. These tests were done at the Dough Rheological Lab., of the Egyptian Center for Bread Technology in El Talbia, Giza, Egypt.

Farinograph test

The Farinograph test was carried out by Farinograph (Model Type No: 81010 ©Brabender® OGH Duisburg, 1979, Germany) to determine the water hydration and mixing properties of each of the supplemented and un-supplemented dough under investigation. The following parameters were determined: water absorption (%), arrival time (min.), dough development time (min.), dough stability (min.), mixing tolerance index (B.U.) and degree of softening (B.U.) as described in the AACC method (AACC, 2011).

Extensograph test

Extensograph test was carried out according to the method described in the AACC method (AACC, 2011) to measure the elastic properties of supplemented and un-supplemented dough. An extensograph (Model Type No: 81010 ©Brabender® OGH Duisburg, 1979, Germany) was used to measure the following parameters: extensograph' E' (mm), resistance to extension 'R' (B.U.), proportional number (R/E ratio) and dough energy (area under the curve, cm^2) of each of the supplemented and un-supplemented doughs under investigation.

TABLE 1. Ingredients of supplemented and un-supplemented pan bread with different percentage of dried OMWW (with and without the addition of %10 lemon juice to the supplemented samples).

Ingredients	Control (-)	1% OMWW		3% OMWW	
	without	without	with	without	with
White flour (72%) (g)	130	128.7	128.7	126.1	126.1
(Whole wheat flour(g	130	128.7	128.7	126.1	126.1
Dry yeast(g)	4	4	4	4	4
Sugar(g)	10	10	10	10	10
Sunflower oil (g)	10	10	10	10	10
Water(g)	170	170	152	170	152
Salt(g)	2.5	2.5	2.5	2.5	2.5
Lemon(mL)	-	-	18	-	18
OMWW(g)	-	2.6	2.6	7.8	7.8

OMWW, olive mill wastewater.

TABLE 2. Ingredients of supplemented and un-supplemented biscuits with different percentage of dried OMWW.

Ingredients	Control (-)	(+) Control TBHQ (200 ppm)	1% OMWW	3% OMWW
White flour (72%) (g)	180	180	178.2	174.6
Egg (g)	50	50	50	50
Sugar(g)	80	80	80	80
Sunflower oil (g)	60	60	60	60
Salt(g)	2	2	2	2
(Baking powder (g	4	4	4	4
OMWW(g)	-	-	1.8	5.4

OMWW, olive mill wastewater; Control (-) is referring to biscuits sample with no added antioxidants, Control (+) is referring to biscuits sample with TBHQ (200 ppm).

Quality characterization of bakery products: (physically and sensory evaluations)

Products physical evaluation

Physical evaluation of the final bakery products of each treatment (un-supplemented and supplemented) were measured according to the following processes:

- *Color evaluation of bakery products*

  Color differences were measured for supplemented and unsupplemented samples by using a spectro-colorimeter (Tristimulus color machine) with CIE lab color scale (hunter, lab scan XE, Reston VA.) calibrated with a white standard tie of Hunter Lab color standard (LX No. 16379):X =77.26, Y = 81.94 and Z = 88.14 (L*= 92.51, a* = -0.88, b* = -0.16) as the method of Giusti et al. (2024) as follows: Subcript (“0” hue) Indicates the color of the control, Hue angle (tan-1 b/a) and saturation index [] were also calculated.

- *Physical change (%) in bakery products (weight, height, area and volume)*

  The evaluation parameters for bread and biscuit samples also included: determination of percentage change in weight after baking (%), height (cm), volume (cm³), index to volume, area (cm2) and tenderness (g/sec.) were done according to Penfield and Campbell (1990). Percentage change in weight after baking was determined for pan bread and biscuits according to the following equation:

$$(\%)\text{ change in weight} = \frac{\text{weight before baking (g)} - \text{weight after baking (g)}}{\text{weight before baking (g)}} \times 100$$

Height (cm), area (cm2) by using a plan meter apparatus, and volume (cm3) by the rapeseed displacement method was determined according to AACC (2011). All measurement were done triplicate.

- *Tenderness of bakery products during storage period*

Degree of tenderness (mm/sec) of Pan bread before and after the storage period (for 7 days at 25°C) and biscuits (for 3 weeks at 25°C) were measured by using a penetrometer apparatus, Model H-1240 with a serial number of 99101240 specs: Ast M, Humboldt MFG, Co., U.S.A., according to Constantin et al. (2020) in the food science lab. at the Nutrition and Food Science Department, Faculty of Home Economics, Helwan University.

Sensory evaluation for bakery products

Sensory evaluation was carried out by 10 trainer panelists (adult individuals) from staff members in the Nutrition and Food Science Department, Faculty of Home Economics, Helwan University, Cairo, Egypt, using a score test of 5-points (1 = lowest quality to 5 = highest quality) according to Stone et al. (2020). The research ethics have been followed: the investigators of the present study took all necessary precautions to minimize risks to participants, especially regarding allergies or food intolerances. They were informed of their right to withdraw from the study at any time without penalty, and maintaining the confidentiality of all participants' data.

Nutritional value of the supplemented bakery products

The prepared product samples (bread, and biscuits) were subjected to chemical analysis to determine nutritional value: Ash, total protein, total fat, and total carbohydrates by difference according to AOAC (2023). Determination of minerals and vitamins were carried out as the following: The prepared product sample (bread, and biscuits), which achieved better properties was subjected to the determination of vitamin E by HPLC according to AOAC (2023). Also, products achieved better properties were subjected to minerals analysis for calcium (Ca) and potassium (K) according to AOAC (2023).

Determination of total phenol contents

Total phenol content was determined in bread, and biscuits using the Folin–Ciocalteu reagent, according to Abdel-Moemin nd Aborya (2014). The test is based on all phenolic compounds contained in the samples being oxidized by the Folin–Ciocalteu reagent.

Thiobarbituric Acid value (TBA) of biscuits during storage period

One gram of biscuits was dissolved in 3.5 mL of cyclohexane, and 4.5 mL of 7.5% trichloroacetic acid (TCA) / 0.34 % TBA was subsequently added. The resulting mixture was shaken for 5 min. After centrifugation (KAIDA, TG18G, China) for 15 min at 2870 g, the TCA-TBA phase was removed and heated in a water bath at 100 °C for 10 min. Absorbance at 532 nm was measured (Pimpa et al., 2009).

Statistical Analysis

Statistical analyses were performed in triplicate except for sensory results, which was conducted with ten replicates, using SPSS V.15.0 for Windows. One-way ANOVA was carried out at ($p < 0.05$).

Results and Discussion

Polyphenol profile of dried OMWW by HPLC, total phenol (TP), and total flavonoids (TF) content of dried OMWW

In this investigation, HPLC analysis was used to determine the polyphenolic profile of dried OMWW, as presented in (Table 3). The results indicated that concentrations of bioactive compounds in dried OMWW included: oleuropein, protocatechuic acid, *p*-hydroxybenzoic acid, chlorogenic acid, syringic acid, caffeic acid, *p*-coumaric acid, and vanillic acid as 235.2, 65.95, 10.21, 7.80, 5.16, 3.76, 1.87, and 1.42 mg/100g, respectively. Among the entire polyphenol compound detected in dried dried OMWW, oleuropein concentration was found to be the highest. According to Karković Marković et al. (2019), all of these phenolic compounds are thought to be bioactive and have a number of advantageous qualities, such as anti-inflammatory, antiviral, anticancer, and antioxidant activities.

The findings of the present study align with the results reported by Benincasa et al. (2022), who found that phenolic compounds identified in DOMWW included oleuropein, caffeic acid, p-coumaric acid, and vanillic acid, with concentrations of 10.3, 0.30, 0.50, and 0.80 mg/100g, respectively. However, the concentration of oleuropein of the present study (235›2 mg/100g) was found to be higher than the value 1.3 mg/100g reported by Benincasa et al. (2022). As well as, Cuffaro et al. (2024), who studied that polyphenol profile of OMWW extract reported that the concentration of caffeic acid, vanillic acid, and oleuropein were 1.62, 0.41, and 3.61 mg/100g. This variation could be due to the differences in the variety of olive mill used in both studies.

Total phenol content and total flavonoid content reported in the present study were 5.749 g GAE/L and 2.417 g CAE/L, respectively. The value of TP obtained in the present study was lower than the value 23.93 g GAE/L reported by El Haimer et al. (2024). However, TP result was higher than the value 0.335 g GAE/L reported by El Abbadi et al. (2024), and lower than the reported values 6.022 g GAE/L by De Bruno et al. (2022). The value of TF obtained in the present study was lower than the value 9.83 g CAE/L reported by Alrowais et al. (2023). A number of factors, such as cultivar differences, climatic circumstances, soil type, fertilizers utilized, irrigation techniques, olive varietals, fruit ripeness, harvest timing, and the extraction processing equipment, can be the reasons for the variability in OMWW's polyphenol profile across various studies.

The impact of dried OMWW with lemon juice on rheological properties of wheat flour

Results of farinograph test

Properties of wheat flour un-supplemented and supplemented with dried OMWW with the addition of lemon juice 10 % according to farinograph parameters are presented in (Table 4 and Fig. 1). Water absorption (%) decreased in all samples supplemented with OMWW compared to the control. These results agree with Dahdah et al. (2024), who studied the effect of olive pomace on the rheological properties of dough bread by farinograph. The investigators stated that water absorption (%) decreased in the supplemented sample compared to the control and reduced gluten formation. This finding is due to the interactions between polyphenols and fiber with water and the starch–gluten matrix. The present study detected a decrease in the water absorption capacity of samples treated with dried OMWW and lemon juice in comparison to samples without the addition of lemon juice and the control. The present results accordance with Gupta et al. (2012), who reported a decrease in water absorption (measured by farinograph) with the addition of citric acid and malic acid at different levels along with the enhancers to the flour. Moreover, the present results agreement with Su et al. (2019) who explained that the presence of organic acids can create an ideal acidic environment for protease activity. This can result in the partial breakdown of gluten, weakening its ability to bind water and reducing its moisture content.

TABLE 3. Polyphenol profile results by HPLC, total phenol (TP) and flavonoids (TF) content of dried OMWW.

Compounds	Values
Protocatechuicacid (mg/100g)	65›95 ±0.39
p-hydroxybenzoic acid (mg/100g)	10›21 ±0.11
Chlorogenic acid (mg/100g)	7.80 ±0.08
Caffeic acid (mg/100g)	3›76 ±0.05
Syringic acid (mg/100g)	5›16 ± 0.07
Vanillic acid (mg/100g)	1›42 ± 0.04
p-coumaric acid (mg/100g)	1›87 ± 0.04
Oleuropein (mg/100g)	235›2 ± 0.61
TP g GAE/L	5.75 ± 0.05
TF g CAE/L	2.42 ± 0.03

Moreover, the results showed that the dough development time of the supplemented wheat flour dough decreased as the percentage of OMWW increased, compared to the control. The reason may relate to the decrease in gluten formation as a partial replacement of wheat flour by dried OMWW, which led to a decrease in flour protein content and a decrease in the disulfide bond that are essential for gluten formation. Investigators (Uthayakumaran et al., 1999) found that the mixing tolerance index decreased in the supplemented sample with OMWW compared to the control. Also, phenolic compounds and antioxidants exist in extract or flour to decrease the disulfide bond which is an essential part of the gluten matrix (Czajkowska–González et al., 2021). Therefore, the degree of softening was the highest in the sample treated with 3% dried OMWW compared to control and other samples.

Results of extensograph test

Rheological properties of wheat flour un-supplemented and supplemented with dried OMWW (with and without lemon juice) according to extensograph tests are presented in (Table 4 and Fig. 2**)**. Resistance degree to extension of the control sample had the lowest value compared to all supplemented samples 320 B.U. While, the supplemented flour with (3% OMWW and lemon juice) sample had the highest resistance degree to extension 720 B.U. Dough extensibility of supplemented samples with dried OMWW decreased compared to the control sample and decreased more in sample with OMWW and lemon. Han and Koh (2011) found that the inclusion of phenolic acids resulted in a reduction in the required mixing time to extension and increased extensibility in the overmixed dough. This dough is likely to have a viscous flow and will be unable to hold its shape. Extensibility of dough had an inverse relationship with its extension to resistance Guo et al. (2021). Also, Dahdah et al. (2024) reported an increase in resistance to extension and a decrease in elasticity due to a decrease in gluten formation.

TABLE 4. Rheological Properties of wheat flour dough supplemented with dried OMWW with lemon juice 10% according to farinograph test and extensograph test in comparison to control and un -supplemented samples without lemon juice.

Samples of wheat flour dough	Farinograph test					
	Water absorption (%)	Arrival time (min)	Dough Development Time (DDT) (min)	Dough Stability (min)	Mixing Tolerance Index (MTI)(B.U)	Degree Softening (B.U)
Control	70.9	1.5	2	10	60	40
1% OMWW	68	1.0	1.5	9.5	40	40
1% OMWW with 10% lemon	62.5	1.5	2	6.5	60	100
3% OMWW	70.5	0.5	1	6	40	140
3% OMWW with 10% lemon	64	2	2.15	7.15	10	100

Samples of wheat flour dough	Extensograph test.			
	Resistance to Extension (Max.) (R) "Elasticity" (B.U.)	Dough Extensibility (E) (mm)	Proportional Number (Max.) R/E **	Dough Energy (cm2)
Control	320	135	2.1	65
1% OMWW	425	115	3.3	68
1% OMWW with 10% lemon	390	100	3.8	50
3% OMWW	580	125	4.16	90
3% OMWW with 10% lemon	720	95	7.3	80

OMWW: olive mill wastewater.

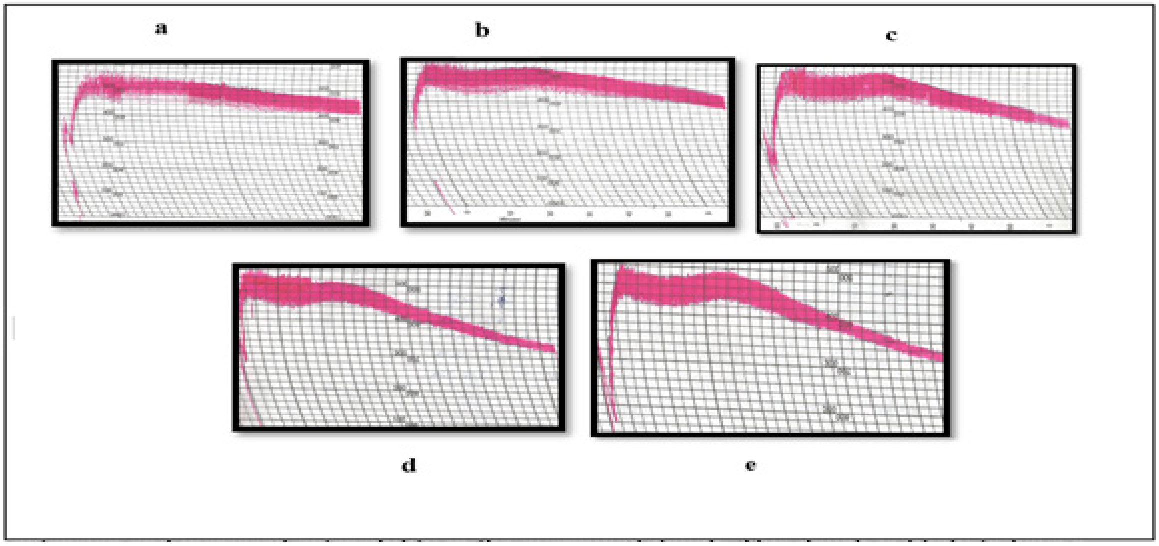

Fig. 1. Properties of wheat flour dough supplemented with dried olive mill wastewater with lemon according to farinograph test, a = control, b= 1% OMWW, c=1%OMWW with lemon, d= 3% OMWW, and e=3%OMWW with lemon.

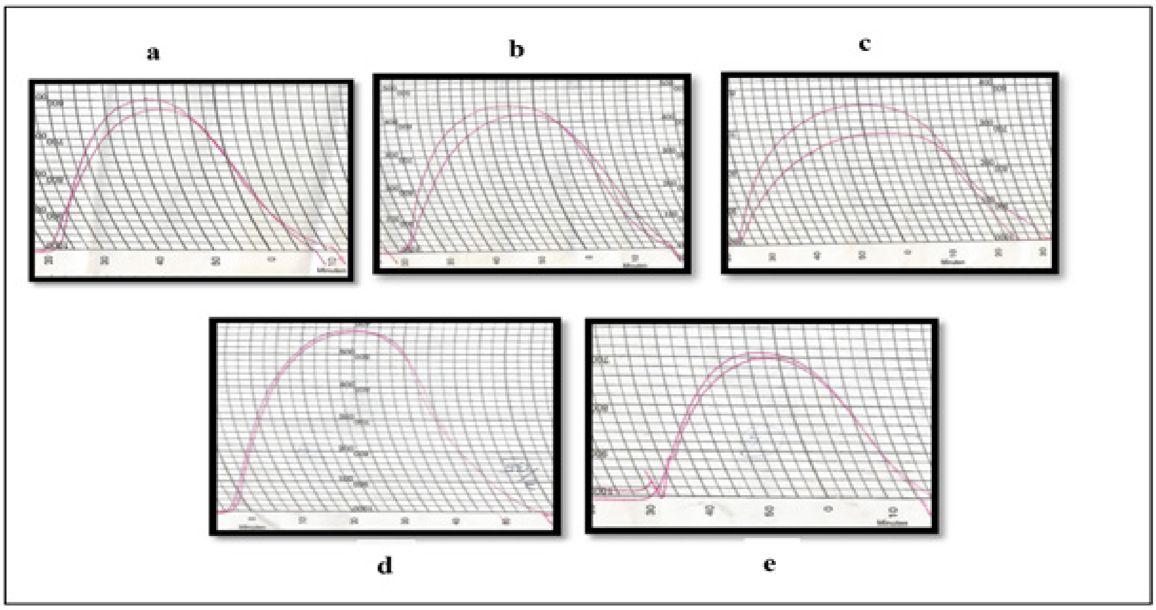

Fig. 2. Properties of wheat flour dough prepared samples according to extensograph test. a = control, b= 1% OMWW, c=1%OMWW with lemon, d= 3% OMWW, and e=3%OMWW with lemon.

Physical properties of pan bread

Physical properties result of pan bread samples (un-supplemented and supplemented) are presented in (Fig. 3). There was no difference existed in water uptake between un-supplemented and supplemented samples ($p > 0.05$). After baking, loafs weight of samples supplemented with OMWW were found to be higher than that of the un-supplemented sample. The present results agreement with the finding of Baiano et al. (2015) who reported that the effect of olive leaf extract on bread weight increased compared to control. This finding as stated by the investigators might be due to the presence of soluble solids in the extracts with a certain water holding capacity that slowed the migration of water from the inside to the outside during baking. Moreover, relative to the control (%) the present study showed that the height of pan bread loaf supplemented with 1% OMWW and 3% OMWW; respectively are higher in comparison to the control. Also, loaf volume and area were found to be higher for both supplemented samples %1 and 3%OMWW than the control.

Physical properties of pan bread supplemented with dried OMWW and with lemon and an un-supplemented sample are presented in (Fig. 3). It was found that no difference between samples existed in percentage of water uptake and fermentation time. Baking time of supplemented samples with OMWW and lemon 33 min decreased compared to un supplemented sample 40 min. Weight of all the prepared samples after baking decreased, while the weight of the supplemented samples with 1% and 3% OMWW with lemon were higher 177.32 and180.75; respectively compared with the control 170.03g.

On the other hand, there was no significant difference ($p < 0.05$) detected in height and index to volume between supplemented and un-supplemented samples. Pan bread samples supplemented with 1% OMWW and 3% OMWW and lemon showed a higher loaf volume 103.652 and 108.989; respectively relative to the control (%). Also, it was found that the volume of the supplemented sample with 3% OMWW and lemon increased slightly compared to the supplemented sample 3% OMWW without lemon and loaf area relative to the control (%), was higher for all the supplemented samples compared to control. The present findings coincided with Blanco et al. (2011) who studied the effect of citric acid on bread and found that loaf volume in bread supplemented with citric acid increased compared to control.

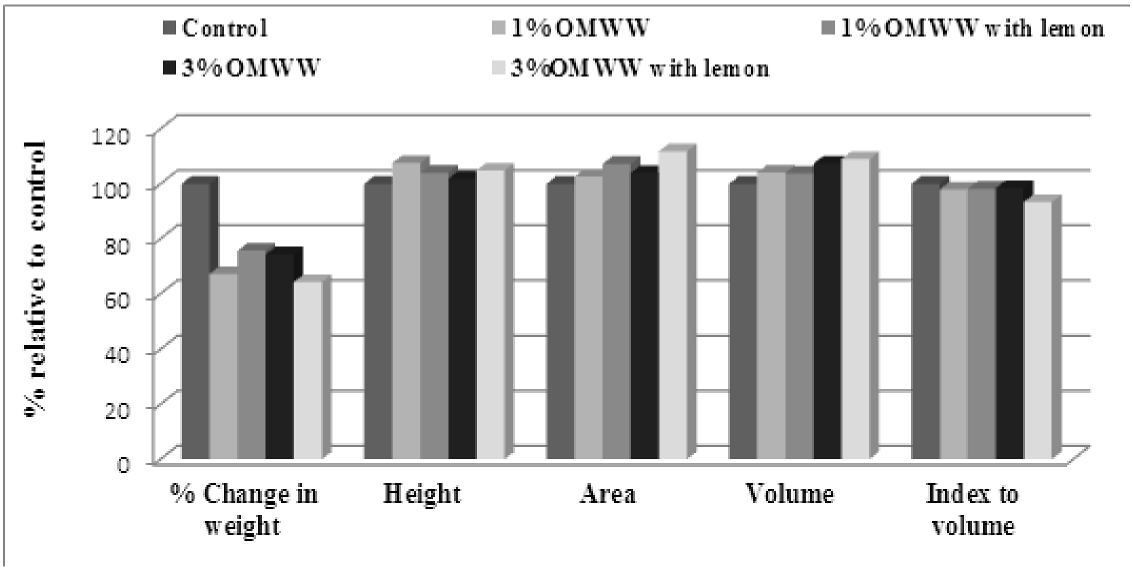

Fig. 3. Physical evaluation (% relative to control) of pan bread prepared with different levels of dried OMWW with and without lemon in comparison to control sample.

Tenderness of pan bread during storage period

Degree of tenderness values of pan bread as indicated by the penetrometer (mm/sec) instrument are presented in (Table 5). It shows that bread prepared with different levels of OMWW with and without lemon at the initial time (after baking) was higher than that of the un-supplemented sample (control). In this context, Conte et al. (2021) studied the effect of phenolic-rich extracts of OMWW on gluten-free breadsticks and found that the hardness decreased with an increased level of OMWW extract in fortified breadsticks compared to the control. The previous study attributed that to the higher moisture content and water activity observed in the samples enriched with the OMWW extract.

After 3 days of storage, the sample supplemented with 1% OMWW with lemon had a higher degree of tenderness than the control and sample 1% OMWW. While, supplemented sample with 3% OMWW had the highest tenderness value among all the other samples. The results of the present study accordance what reported by other investigators (Altamirano-Fortoul et al., 2015; Su et al., 2019), who found that different organic acids have an effect on the properties of bread. Addition of organic acids to the bread caused a crumb softening effect on the fresh bread 0 d as well as during storage for 1 d, 3 d, and 5 d, and consequently delayed the short-term retrogradation of the bread due to low pH value.

TABLE 5. Mean values of tenderness (mm/sec) of pan bread prepared samples with different levels of dried olive mill wastewater and the control under storage conditions (25ºC up to 7 days).

Storage period	Control	1% OMWW	1% OMWW with lemon	3% OMWW	3% OMWW with lemon
(Zero time (after baking	9.33 ± 2.1[c]	17.67 ± 1.5[b]	16.33 ± 0.6[a]	20.67 ± 2.5[b]	19.33 ± 2.1[a]
3 Days	2.67 ± 0.6[b]	16.67 ± 2.5[a]	12.67 ± 2.1[a]	17.0 ± 2.0[a]	15.67 ± 3.1[a]
7 Days	1.33 ± 0.6[c]	3.67 ± 1.5 [c]	4.37 ± 2.5[ab]	7.00 ± 1.0[b]	7.33 ± 1.5 [a]

Different lowercase letters in the same row indicate that there are statistically significant differences between the means, with a significance level of ($p < 0.05$), Data were presented as (mean ± SD), OMWW: olive mill wastewater.

The present study showed that after 7 days of storage conditions at (25 °C), there were significant decreases ($p < 0.05$) in bread tenderness of all treated samples. While, the control and 1% OMWW samples had a lower degree of tenderness than the other supplemented pan bread sample with 3% OMWW with and without lemon juice as well as the supplemented sample with 1% OMWW with lemon.

Sensory evaluation of pan bread

The appearance and texture of baked goods are crucial factors that determine their overall quality, and subsequently, have a significant impact on consumer preferences. The sensory evaluation (score) for the prepared pan bread with different levels of dried OMWW (without and with lemon) are presented in (Fig. 4) in comparison to the control sample. It was found that pan bread supplemented with 1% OMWW had higher significant ($p < 0.05$) scores for the following evaluated characteristics: odor, taste, flavor, crust color, external color, and general acceptability than that of the supplemented with 3% OMWW. It is worth noting that during sensory evaluation, panelists sensed bitterness taste in sample supplemented with a high percentage of OMWW (3%). This observation is due to polyphenols content in OMWW. According to Cedola et al. (2019), most polyphenols obtained from olive by-products sources cause an astringent or bitter taste in bread. In another study (Cedola et al., 2020) investigator reported that adding OMWW and OP to bread and pasta showed decreases in products characteristics compared to the control sample with regard to: color, odor, taste, crust firmness, crumb firmness, large bubbles and overall quality.

Moreover, the results of the present study showed no significant differences ($p < 0.05$) scores between the control sample and supplemented samples 1% OMWW and 3% OMWW for the cell uniformity characteristic. However, tenderness property increased significantly ($p < 0.05$) in the supplemented sample with OMWW with and without lemon juice compared to the control sample. There was no significant differences ($p < 0.05$) scores between the control sample and 1% OMWW with lemon sample with regard to appearance, odor, taste, and crust color. However, there was a significant decrease ($p < 0.05$) in the score of following sensory properties: (flavor, interior color and cell uniformity), between supplemented samples with lemon juice 1%OMWW and 3% OMWW and the control sample.

It was concluded from (Fig. 4) that the sensory characteristics of pan bread supplemented with OMWW for each sample with lemon juice 1% OMWW and 3% had a better score than the supplemented samples without lemon 1% OMWW and 3% OMWW. Addition of lemon juice to the supplemented sample with OMWW enhanced the product's sensory characteristics. Lemon juice as a source of citric acid acted as a mask to cover the feeling of bitterness taste and enhances the sensory evaluation of the supplemented samples with OMWW. Zhao et al. (2020) studied the addition of citric acid at different levels in steamed bread, increased the aroma score, but the aroma score decreased as the level of citric acid increased.

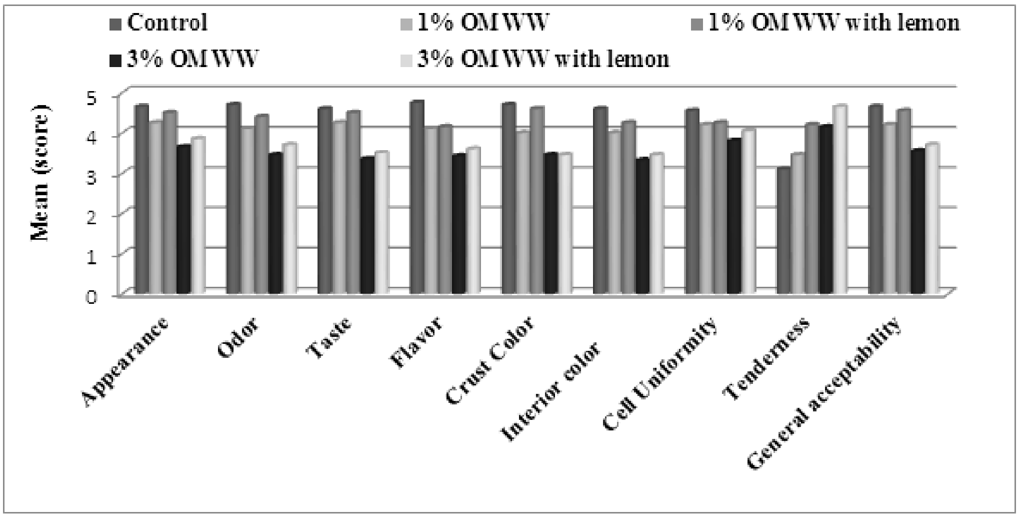

Fig. 4. Sensory evaluation (score) of pan bread supplemented with different levels of dried olive mill wastewater (with and without lemon juice) in comparison to control sample.
OMWW: olive mill wastewater.

Color evaluation of pan bread

Hunter colorimeter results of pan bread supplemented with different levels of dried OMWW are presented in (Table 6 and Fig. 5). It was found that the external crust color of pan bread supplemented with OMWW showed a decrease in lightness (L) value especially with an increased level of OMWW compared to the control, which showed more lightness (L) color value 63.94. On the other hand, redness (a) values increased in all the supplemented bread samples compared to the un-supplemented sample (control) except for 1% OMWW supplemented sample which showed better color. As observed, values for yellowness (b) were slightly lower in the sample with 3% OMWW than that of the control, while the value for yellowness (b) in the control sample equal 1% OMWW supplemented sample 30.63.

The above results agreement with Baiano et al. (2015) who determined the color of the crumb of bread supplemented with olive leaf extract and found that the lightness (L) value lower than that of the control sample, while the redness (a) and yellowness (b) values higher than the control values. On the contrary, the results of Conte et al. (2021) disagreements with the results of the present study, who found that no significant differences $p < 0.05$ in lightness (L) values between supplemented bread with OMWW extract and control. While, redness (a) and yellowness (b) values decreased compared to the control. Saturation index was related to Hunter (a) and (b) values, which indicated degrees of color intensity, the present results indicated that the saturation value for pan bread supplemented with 3% OMWW had the highest value compared to the control and 1% OMWW. Also, results showed that hue angle values for the control and 1% OMWW were close in values, while the hue angle value increased in 3% OMWW sample.

Table 6. Hunter color values for crust of pan bread prepared supplemented with different levels of dried olive mill wastewater and the control sample

Bread Sample	L*	a*	b*	$^{a}/_{b}$	Saturation index	Hue angle
Control	63.94 ± 0.2	9.91 ± 0.2	30.63 ± 0.1	0.32	32.19	72.07
1% OMWW	63.47 ± 0.1	9.89 ± 0.1	30.63 ± 0.1	0.32	32.18	72.10
3% OMWW	61.05 ± 0.1	11.95 ± 0.1	32.09 ± 0.04	0.37	34.24	69.57

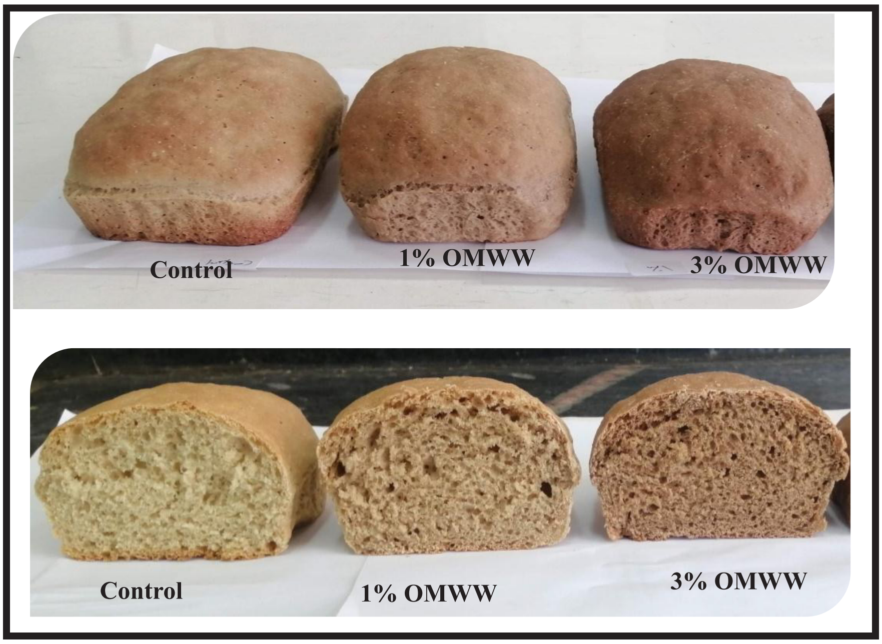

Fig. 5. Photograph image of pan bread prepared supplemented with different percentages of dried olive mill wastewater after baking .
OMWW: olive mill wastewater

Nutritional value of pan bread

The nutritional value of supplemented pan bread samples with different levels of dried OMWW with lemon juice are presented in (Table 7). Results showed that no significant differences (p < 0.05) between the supplemented and un-supplemented bread sample. It was found that there was a slight increase in carbohydrates and ash content with an increased level of OMWW compared to the control. Results showed agreement with results of Di Nunzio et al. (2020), who studied the effect of defatted olive by-products on conventional fermented bread and sourdough fermented bread. The investigators found an increase in the ash content of the supplemented bread with defatted olive by-products.

Minerals content of pan bread prepared with dried OMWW with lemon are presented in (Table 7). It was found that 3% OMWW bread sample content of calcium and potassium increased 380, ٥٧0 mg /100g; respectively compared to the control sample ١٢0, 490 mg /100g; respectively. This is due to the high calcium and potassium content of OMWW as reported previously by Alrowais et al. (2023). Tocopherol content of pan bread supplemented with OMWW with lemon was found to be higher in 3% OMWW sample than that of the control sample. Di Nunzio et al. (2020) found that the conventional fermented bread 4% sample supplemented with defatted olive by-products had a higher tocopherol content than conventional fermented bread 0% sample un-supplemented.

Physical properties of biscuits

Physical properties result of supplemented biscuits with different levels of dried olive mill wastewater as well as the control samples are presented in (Table 8). Weight after baking increased in the supplemented samples: 1% OMWW and 3% OMWW 6.05 and6.21 g; respectively compared to the control samples with and without antioxidant samples 6.01 and 6.03 g; respectively. According to Gysel et al. (2004), this finding might be due to the hygroscopicity properties of OMWW led to the holding capacity of water in biscuits, then the weight increased in supplemented samples due to the existences of soluble minerals**.** The present study showed no significant difference (p < 0.05) found between biscuits supplemented samples for volume it ranged from 65.33-66.32 cm^3 compared to control without and with antioxidants 67.66 and 65.66 cm^3 ; respectively. Also, results showed that the difference was not significant at (p < 0.05) between supplemented biscuits samples for area (cm^2) compared to control with and without antioxidant 16.66 and16.80 cm^2; respectively. On the other hand, there was a significant difference at (p < 0.05) in height between biscuits supplemented samples of 1% OMWW and 3% OMWW 0.67 and 0.66 cm; respectively which decreased compared to control without antioxidant 0.700 cm.

TABLE 7. Nutritional value, minerals and tocopherol content of suplemented pan bread with different levels of dried olive mill wastewater with lemon

Samples*	Nutritional value (%)				Minerals and Tocopherol content (mg/100g)		
	Protein	Fat	Carbohydrate	Ash	Ca	K	Tocopherol
Control	12.73 ±0.6 [a]	3.16 ±0.1 [a]	82.22± 3.7 a	1.56 ±0.1 [a]	120 ± 2.6	490 ± 2.6	1.4 ± 0.3
1% OMWW with lemon	12.64 ±0.6 [a]	3.07 ±0.1 [a]	82.32 ± 3.7 [a]	1.64 ±0.1a	ND	ND	ND
3% OMWW with lemon	12.53 ±0.6 [a]	3.11 ±0.1 [a]	82.37± 3.7 a	1.66 ±0.1 [a]	380 ± 6.6	570 ± 5.6	15.3 ± 0.4

Different lowercase letters in the same column indicate that there are statistically significant differences between the means, with a significance level of $p < 0.05$. OMWW: olive mill wastewater. Data were presented as (mean ± SD), ND: Not determined

TABLE 8. Mean values of physical properties of supplemented biscuits with different levels of dried olive mill wastewater and the control samples $^{(-\ and +)}$.

Physical properties	unit	Control $^{(-)}$	Control $^{(+)}$	1% OMWW	3% OMWW
Baking time	min	33	34	39	44
Weight before baking	g	7	7	7	7
Weight after baking	g	6.01	6.03	6.05	6.21
% Change in weight	%	14.14	13.86	13.57	11.29
Height	cm	0.70 ± ٠,٠²a	0.68 ± ٠,٠٢ [ab]	0.67 ± 0.01 [ab]	0.66 ± 0.11 [b]
Area	c^m2	16.66 ±0.25 [a]	16.80 ± 0.26 [a]	15.60 ± 0. 2 [a]	15.90 ± 1.12 [a]
Volume	c^m3	67.66 ± 2.0⁸a	65.66 ± 4.9³a	65.33 ± 3.79 [a]	66.32 ± 2.83 [a]
Index to volume	cm	4.44 ± 0.03 [ab]	4.65 ± 0.06 [a]	4.50 ± 0.07 [a]	4.31 ± 0.13 b

Different lowercase letters in the same row indicate that there are statistically significant differences between the means, with a significance level of $p < 0.05$. Data were presented as (mean ± SD). OMWW: olive mill wastewater, Control $^{(-)}$ is referring to biscuits sample with no added antioxidants, Control $^{(+)}$ is referring to biscuits sample with TBHQ (200 ppm).

Tenderness of biscuits during storage period

Degree of tenderness of the supplemented biscuits with different levels of dried OMWW measured by a penetrometer instrument (mm/sec) at the initial time (after baking) and upon storage period for 3 weeks at room temperature (25 ºC) is presented in (Fig. 6). The results showed that there was a significant increase in degree of tenderness ($p < 0.05$) of biscuit-supplemented with 1% OMWW and 3% OMWW and un-supplemented samples at zero time and during storage period for 3 weeks. Results of the present study correspond with Paciulli et al. (2023) who studied the effect of olive leaves extract on the hardness of biscuits and found that the hardness of supplemented samples with olive leaves extract decreased significantly ($p < 0.05$) compared to the control. The previous study reported that the hardness of biscuits enriched with onion residue decreased due to breakdown of the gluten matrix (Jiang et al., 2021).

Total phenol content of bakery products

Total phenol (TP) content of un-supplemented and supplemented bakery products with dried OMWW are shown in (Table 9). Results indicated that TP increased significantly ($p < 0.05$) in the 3%

OMWW bread sample compared to the control. In addition, these results coincided with the presented results in (Table 3). These results agreed with Pampuri et al. (2021), who studied the effect of OMWW on gluten-free breadsticks, biscuits, and mayonnaise, the investigators stated that there was an increase in the TP content of the samples compared to the control. Phenolic compounds are the main antioxidant elements found in dried OMWW (Benincasa et al., 2022; Cuffaro et al., 2023). The principal phenolic compounds are hydroxytyrosol, oleuropein, tyrosol, caffeic acid, p-coumaric acid, vanillic acid, verbascoside, elenolic acid, catechol, and rutin.

Sensory evaluation of biscuits

Sensory evaluation results (score) for supplemented biscuits with different levels of dried OMWW are shown in (Table 10). It was found that the score values for odor, taste, tenderness, and texture (softness) of supplemented biscuits (with 1% OMWW and 3% OMWW) were not differ significantly at ($p < 0.05$) compared to the control$^-$ without antioxidants (4.60, 4.60, 4.05, and 4.45; respectively) and control$^+$ with TBHQ. Results of the present study accordance with Palmeri et al. (2019), who reported that no significant differences ($p < 0.05$) in scores for taste were detected between fortified fresh pasteurized milk sample with olive leaf extract and unfortified milk**.** The present study detected a significant decrease ($p < 0.05$) in appearance, flavor, color, and general acceptability of supplemented biscuits 1% OMWW and 3% OMWW samples compared to the control $^{(-)}$ (without antioxidants). Results agreement with Bolek (2020) reported that color and overall impression decreased (score) significant at ($p < 0.05$) with an increased level of olive stone. Moreover, Faccioli et al. (2021) reported that when increasing levels of olive leaves in crackers negatively influenced in appearance, taste, aroma, and texture.Panelists felt slightly bitter after taste for the supplemented samples with 3% OMWW. This feeling can be solved by adding a little sugar and fat to the product, which made a mask to overcome the bitter taste. Omar (2010) reported that oleuropein is the compound responsible for the bitter taste of olive leaf flour. Also, it was found that changing in color from gold to brown led to decreased general acceptability.

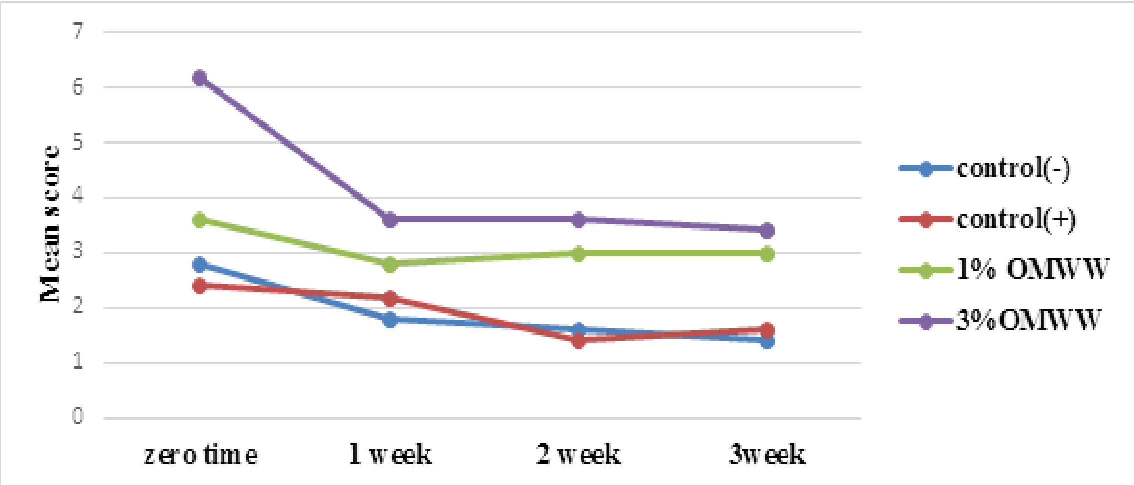

Fig. 6. Tenderness (mm/sec) as mean values of supplemented biscuits with different levels of dried olive mill wastewater and control samples $^{(-\text{ and }+)}$ under storage conditions (25°C up to 3 weeks).
(OMWW: olive mill wastewater, Control $^{(-)}$ is referring to biscuits sample with no added antioxidants, Control $^{(+)}$ is referring to biscuits sample with TBHQ (200 ppm).

TABLE 9. Total phenol content (mg/100 g GAE) of bakery products (bread and biscuit) supplemented with dried olive mill wastewater .

Products	Control $^{(-)}$	3% OMWW
Pan bread	50.10 ± 0.01^b	52.46 ± 0.59^a
Biscuits	51.79 ± 0.33^b	53.71 ± 1.03^a

Different lowercase letters in the same row indicate that there are statistically significant differences between the means, with a significance level of $p < 0.05$. Data were presented as (mean ± SD). OMWW: olive mill wastewater.

TABLE 10. Mean values (score) of sensory characteristics of supplemented biscuits with different levels of dried olive mill wastewater and the control samples (- and+)

Characteristics	Appearance	Odor	Taste	Tenderness	Flavor	Color	Texture of softness	General acceptability
Control (-)	4.75 ± 0.42ᵃ	4.60 ± 0.46 ᵃ	4.60 ± 0.46 ᵃ	4.05 ± 0.52 ᵃ	4.65 ± 0.47 ᵃ	4.75 ± 0.42 ᵃ	4.45 ±0.50 ᵃ	4.45 ± 0.50 ᵃ
Control (+)	4.70 ± 0.42ᵃ	4.50 ± 0.47ᵃ	4.50 ± 0.47 ᵃ	4.95 ± 0.49ᵃ	4.45 ±0.50 ᵃᵇ	4.55 ± 0.50 ᵃ	4.45 ± 0.50 ᵃ	4.45± 0.50 ᵃ
1% OMWW	4.10 ± 0.84ᵇ	4.10 ± 0.93 ᵃ	4.15 ± 0.71 ᵃ	4.75 ± 0.28ᵃ	4.00± 0.67ᵇᶜ	4.05± 0.50 ᵇ	4.2 ± 0.59ᵃ	4.15 ±0.71ᵃᵇ
3% OMWW	3.60 ± 0.52ᵇ	4.00 ± 0.53 ᵃ	4.05 ± 0.64ᵃ	4.90 ± 0.72ᵃ	3.85 ± 0.53ᶜ	3.75 ± 0.68 ᵇ	3.9 ± 0.74ᵃ	3.75 ± 0.79ᵇ

Different lowercase letters in the same row indicate that there are statistically significant differences between the means, with a significance level of $p < 0.05$. Data were presented as (mean ± SD). OMWW: olive mill wastewater. Control (-) is referring to biscuits sample with no antioxidants, Control (+) is referring to biscuits sample with TBHQ (200 ppm).

Oxidative stability of biscuits (by using Thiobarbituric acid test) during the storage period

Thiobarbituric acid was employed to assess the oxidative stability of biscuits during the storage period. Unlike hydroperoxides, malonaldehyde, a secondary lipid-oxidation product, was measured in this study. The non-antioxidant samples displayed the highest TBARS rates, indicating elevated malonaldehyde levels and increased secondary oxidation levels in the sample (Passos et al., 2019). Thiobarbituric acid values of supplemented biscuits without and with different levels of OMWW during storage at (25 ± 1ºC for 3 weeks) are presented in (Table 11). Results showed that there were no significant differences excited at (p <0.05) between supplemented biscuits with and without OMWW after the first and second week. Nevertheless, after 2 weeks and 3 weeks supplemented biscuits with OMWW decreased in thiobarbituric acid values as the levels of dried OMWW increased, compared to the control⁻ sample without additives. While control⁺ with TBHQ had the lowest value in thiobarbituric acid compared to the other samples. Paciulli et al. (2023) observed that biscuits samples treated with the olive leaves extract after a storage period for 5 days decreased significantly in lipid oxidation compared to the control. Indeed, it could be observed that the phenolic compounds of olive leaves extract led to a decrease in secondary lipid-oxidation products and extended the shelf life of biscuits.

Color evaluation of biscuits

Hunter colorimeter results for biscuits supplemented with different levels of dried olive mill wastewater are presented in (Table 12 and fig. 7). It was found that the lightness (L) values of the supplemented biscuit samples with OMWW decreased compared to control(-) 60.37 and control(+) 59.25. Results showed that the redness (a) value of (3%OMWW) (13.31) increased compared to the control(-) value 11.30, while the redness (a) value of 1% OMWW was close to the control (-) value 11.30. However, the yellowness (b) value was lower than compared to the control (-), whereas the yellowness value of 3% OMWW sample was 29.85 close to the control(-) value 30.47. The observed results were in line with (Bolek, 2020; Paciulli et al., 2023). Ouarouer et al. (2019) reported that when polyphenols are oxidized to quinone form, they can undergo reactions with amino acids and proteins, specifically the Maillard reaction; this would therefore lead to an increase in surface darkness. Saturation index was related to Hunter (a) and (b), values which indicated degrees of color intensity. Results indicated that the value of (3% OMWW) supplemented sample was 32.68 close to the control(-) value 32.50, while the saturation index decreased in the control(+) and 1% OMWW sample 31.70 and 29.21; respectively compared to the control(-). In addition to, Hue values decreased in 1% OMWW and 3% OMWW supplemented samples compared to control(+) and control(-) 70.65 and 69.65; respectively.

TABLE 11. Mean values of Thiobarbituric acid for supplemented biscuits with different levels of dried olive mill wastewater and control samples during storage (at 25±1°C for 3 weeks).

Biscuits samples	Zero time	1st week	2nd week	3rd week
Control (-)	0.44 ± 0.02 a	0.48 ± 0.02a	0.53 ± 0.02a	0.59 ± 0.02a
Control (+)	0.43 ± 0.02a	0.45 ± 0.02a	0.47 ± 0.02b	0.49 ± 0.02c
1% OMWW	0.44 ± 0.02a	0.48 ± 0.02a	0.50 ± 0.02ab	0.54 ± 0.02b
3% OMWW	0.44 ± 0.02a	0.47 ± 0.02a	0.49 ± 0.02 ab	0.53 ± 0.02bc

Different lowercase letters in the same column indicate that there are statistically significant differences between the means, with a significance level of $p < 0.05$. Data were presented as (mean ± SD).
OMWW: olive mill wastewater, Control (–) is referring to biscuits sample with no added antioxidants, Control (+) is referring to biscuits sample with TBHQ (200 ppm)

TABLE 12. Hunter color values of supplemented biscuits with different levels of dried olive mill wastewater and the control samples.

Biscuits Sample	**L***	**a***	**b***	**a/b**	**Saturation index**	**Hue angle**
Control (-)	60.37 ± 0.04	11.30 ± 0.03	30.47 ± 0.03	0.371 ± 0.03	32.50 ± 0.04	69.65 ± 0.03
Control (+)	59.25 ± 0.03	10.51 ± 0.02	29.92 ± 0.04	0.351 ± 0.03	31.70 ± 0.02	70.65 ± 0.05
1% OMWW	57.63 ± 0.07	11.10 ± 0.05	27.02 ± 0.03	0.411 ± 0.04	29.21 ± 0.04	67.67 ± 0.03
3% OMWW	50.63 ± 0.07	13.31 ± 0.05	29.85 ± 0.04	0.446 ± 0.05	32.68 ± 0.03	65.97 ± 0.04

OMWW: olive mill wastewater, Control (–) is referring to biscuits sample with no added antioxidants, Control (+) is referring to biscuits sample with TBHQ (200 ppm). Data were presented as (mean ± SD).

Control (-) **Control** (+) 1% OMWW 3% OMWW

Fig. 7. photograph image of biscuits prepared supplemented with different percentages of dried olive mill wastewater after baking.
OMWW: olive mill wastewater, Control (–) is referring to biscuits sample with no added antioxidants, Control (+) is referring to biscuits sample with TBHQ (200 ppm).

Nutritional value of biscuits

Nutritional value of supplemented biscuits with different levels of OMWW is presented in (Table 13). Results indicated that no significant differences (p <0.05) were found in protein and fat content among supplemented (1% OMWW and 3% OMWW) samples and control biscuit samples. On the other hand, there were significant increase (p <0.05) in ash content at supplemented 1% OMWW and 3% OMWW samples 0.65 and 0.68; respectively compared to control$^{(+)}$ and control$^{(-)}$0.55 and 0.54; respectively. Also, it was found that supplemented samples with 1% OMWW and 3% OMWW had higher values than control$^{(+)}$ and control$^{(-)}$in carbohydrate content. Results of the present study agreement with Di Nunzio et al. (2020), in ash and carbohydrate contents of the biscuits 2.5% sample increased compared to control. While, the present results disagree with Faccioli et al. (2021) who reported that there were no significant differences between supplemented and un-supplemented crackers which prepared with the addition of olive leaf flour in the ash and carbohydrates content, due to the nutritional value of the OMWW in the present study.

Minerals and Tocopherol content of biscuits

The minerals content of supplemented biscuits with OMWW are presented in (Table 13). It was found that the supplemented biscuits with 3% OMWW had an increase in calcium content 40 mg/100g compared to the control $^{(-)}$ 18 mg/100g. Also, it noted that biscuits supplemented with 3%OMWW contained the highest value of potassium (mg/100g) compared to control $^{(-)}$. Results illustrated that tocopherol content in biscuits supplemented with 3%OMWW increase 12.2 mg/100g compared to control$^{(-)}$ 5.8 mg/100g.

Table 13 Nutritional value, minerals content and tocopherol of supplemented biscuits with different levels of dried olive mill wastewater as dry weight and the control samples

Samples*	Nutritional value (%)				Minerals and Tocopherol contents (mg/100g)		
	Protein	Fat	Carbohydrate	Ash	Ca	K	Vitamin E
Control $^{(-)}$	7.31 ± 0.3 ^{a}	21.33 ±1.1 ^{a}	70.81 ± 0.7 ^{b}	0.54 ± 0.01^{b}	18 ± 2.0	240 ± 3.6	5.8 ± 0.3
Control $^{(+)}$	7.32 ± 0.4 ^{a}	21.39 ± 1.1 ^{a}	70.70 ± 0.7 ^{b}	0.55 ± 0.01^{b}	-	-	-
1% OMWW	7.19 ± ٠,٤ ^{a}	20.89 ±1.0 ^{a}	71.27 ± 0.7^{ab}	0.65 ± 0.02 ^{a}	-	-	-
3% OMWW	6.83 ± 0.3^{a}	20.21 ± 1.0 ^{a}	72.28 ±0.7 ^{a}	0.68 ± 0.02 ^{a}	40 ± 4.4	340 ± 3.6	12.2 ±0.2

Different lowercase letters in the same row indicate that there are statistically significant differences between the means, with a significance level of $p < 0.05$. Data were presented as (mean ± SD).
OMWW: olive mill wastewater, Control (–) is referring to biscuits sample with no added antioxidants, Control (+) is referring to biscuits sample with TBHQ (200 ppm).

Conclusions

The results of this study concluded that addition of dried OMWW to bakery products increased the total phenol content, nutritional value, and oxidative stability of biscuits. Sensory properties of pan bread were enhanced by the addition of dried OMWW and lemon juice (citric acid). However, the color and general acceptability of biscuits decreased in samples with dried OMWW compared to the control, both with and without added antioxidants. This research recommends that the food industry consider enriching bakery products with dried OMWW as functional products, which may introduce bioactive compounds to the products such as total phenols, tocopherols (as antioxidants), calcium, and potassium. Further studies are advised to examine how the properties of other food items are affected by increased levels of wastewater from olive mills.

Acknowledgments

This paper is based upon work supported by Science, Technology & Innovation Funding Authority (STDF) under grant. (Number of funding: 48568).

References

AACC (2011) American Association of Cereal Chemists. Approved methods of the American Association of Cereal Chemists (11th ed.) USA.

Abdel-Moemin, A. R. and Aborya, A. O. (2014) Innovative products of coffee and tea based dandelion roots and leaves (*Taraxacum officinale*). *Agricultura*, **11**, 1-10. https://www.academia.edu/download/37557265/Aly_R_ABDEL-MOEMIN_beverages_distribucija_June_2014.pdf

Alrowais, R., Yousef, R. S., Ahmed, O. K., Mahmoud-Aly, M., Abdel daiem, M. M. and Said, N. (2023) Enhanced detoxification methods for the safe reuse of treated olive mill wastewater in irrigation. *Environmental Sciences Europe*, **35**(1), 95. https://doi.org/10.1186/s12302-023-00797-2

Altamirano-Fortoul, R., Hernández-Muñoz, P., Hernando, I. and Rosell, C. M. (2015) Mechanical, microstructure and permeability properties of a model bread crust: Effect of different food additives. *Journal of Food Engineering*, **163**, 25-31. https://doi.org/10.1016/j.jfoodeng.2015.04.019

AOAC, (2023) Official methods of analysis, 22nd ed., (online). AOAC International, Rockville, MD.

Arranz-Otaegui, A., Gonzalez Carretero, L., Ramsey, M. N., Fuller, D. Q. and Richter, T. (2018) Archaeobotanical evidence reveals the origins of bread 14,400 years ago in northeastern Jordan. *Proceedings of the National Academy of Sciences*, **115**(31), 7925-7930. http://dx.doi.org/10.1073/pnas.1801071115PMid:30012614

Azzam, M. O. and Hazaimeh, S. A. (2021) Olive mill wastewater treatment and valorization by extraction/concentration of hydroxytyrosol and other natural phenols. *Process Safety and Environmental Protection*, **148**, 495-523. https://doi.org/10.1016/j.psep.2020.10.030

Baiano, A., Viggiani, I., Terracone, C., Romaniello, R. and Nobile, M. D. (2015) Physical and sensory properties of bread enriched with phenolic aqueous extracts from vegetable wastes. *Food Technology* 0.05. Data were presented as (mean ± SD).

Banias, G., Achillas, C., Vlachokostas, C., Moussiopoulos, N. and Stefanou, M. (2017) Environmental impacts in the life cycle of olive oil: a literature review. *Journal of the Science of Food and Agriculture*, **97**(6), 1686-1697. https://doi.org/10.1002/jsfa.8143

Benincasa, C., Pellegrino, M., Romano, E., Claps, S., Fallara, C. and Perri, E. (2022) Qualitative and quantitative analysis of phenolic compounds in spray-dried olive mill wastewater. *Frontiers in Nutrition*, **8**, 782693. https://doi.org/10.3389/fnut.2021.782693

Blanco, C. A., Ronda, F., Pérez, B. and Pando, V. (2011) Improving gluten-free bread quality by enrichment with acidic food additives. *Food Chemistry*, **127**(3), 1204-1209. https://doi.org/10.1016/j.foodchem.2011.01.127

Bolek, S. (2020) Olive stone powder: A potential source of fiber and antioxidant and its effect on the rheological characteristics of biscuit dough and quality. *Innovative Food Science & Emerging Technologies*, **64**, 102423. https://doi.org/10.1016/j.ifset.2020.102423

Cedola, A., Cardinali, A., D'Antuono, I., Conte, A. and Del Nobile, M. A. (2020) Cereal foods fortified with by-products from the olive oil industry. *Food Bioscience*, **33**, 100490. https://doi.org/10.1016/j.fbio.2019.100490

Cedola, A., Cardinali, A., Del Nobile, M. A. and Conte, A. (2019) Enrichment of bread with olive oil industrial by-product. *Journal of Agricultural Science and Technology*, B, **9**, 119-127. doi: 10.17265/2161-6264/2019.02.005

Chaple, S., Sarangapani, C., Dickson, S. and Bourke, P. (2023) Product development and X-Ray microtomography of a traditional white pan bread from plasma functionalized flour. *Lwt,* **174**: 114326. https://doi.org/10.1016/j.lwt.2022.114326

Constantin, G. A., Voicu, G., Stefan, E. M, Tudor, P., Paraschiv, G. and Munteanu, M. G. (2020) *International Journal of Food Sciences and Nutrition,* **14**(9): 117-122

Conte, P., Pulina, S., Del Caro, A., Fadda, C., Urgeghe, P. P., De Bruno, A., Difonzo, G., Caponio, F., Romeo, R. and Piga, A. (2021) Gluten-free breadsticks fortified with phenolic-rich extracts from olive leaves and olive mill wastewater. *Foods*, **10**(5), 923. https://doi.org/10.3390/foods10050923

Cuffaro, D., Bertolini, A., Bertini, S., Ricci, C., Cascone, M. G., Danti, S., Saba, A., Macchia, M. and Digiacomo, M. (2023) Olive Mill Wastewater as Source of Polyphenols with Nutraceutical Properties. *Nutrients*, **15**(17), 3746. https://doi.org/10.3390/nu15173746

Cuffaro, D., Bertolini, A., Silva, A. M., Rodrigues, F., Gabbia, D., De Martin, S., Saba, A., Bertini, S., Digiacomo M. and Macchia, M. (2024) Comparative Analysis on Polyphenolic Composition of Different Olive Mill Wastewater and Related Extra Virgin Olive Oil Extracts and Evaluation of Nutraceutical Properties by Cell-Based Studies. *Foods*, **13**(20), 3312. https://doi.org/10.3390/foods13203312

Czajkowska–González, Y. A., Alvarez–Parrilla, E., del Rocío Martínez–Ruiz, N., Vázquez–Flores, A. A., Gaytán–Martínez, M. and de la Rosa, L. A. (2021) Addition of phenolic compounds to bread: antioxidant benefits and impact on food structure and sensory characteristics. *Food Production, Processing and Nutrition*, **3**, 1-12. https://doi.org/10.1186/s43014-021-00068-8

Dahdah, P., Cabizza, R., Farbo, M. G., Fadda, C., Mara, A., Hassoun, G. and Piga, A. (2024) Improving the rheological properties of dough obtained by partial substitution of wheat flour with freeze-dried olive pomace. *Foods*, **13**(3), 478. https://doi.org/10.3390/foods13030478

De Bruno, A., Gattuso, A., Romeo, R., Santacaterina, S. and Piscopo, A. (2022) Functional and Sustainable Application of Natural Antioxidant Extract Recovered from Olive Mill Wastewater on Shelf-Life Extension of "Basil Pesto". *Applied Sciences*, **12**(21), 10965. https://doi.org/10.3390/app122110965

Di Nunzio, M., Picone, G., Pasini, F., Chiarello, E., Caboni, M. F., Capozzi, F., Gianotti, A. and Bordoni, A. (2020) Olive oil by-product as functional ingredient in bakery products. Influence of processing and evaluation of biological effects. *Food Research International*, **131**, 108940. https://doi.org/10.1016/j.foodres.2019.108940

Difonzo, G., Troilo, M., Squeo, G., Pasqualone, A. and Caponio, F. (2021) Functional compounds from olive pomace to obtain high‐added value foods–a review. *Journal of the Science of Food and Agriculture*, **101**(1), 15-26. https://doi.org/10.1002/jsfa.10478

El Abbadi, S., El Moustansiri, H., Douma, M., Bouazizi, A., Arfoy, B., Calvo, J. I. and Tijani, N. (2024) Enhancing the performance of alumina-pillared clay for phenol removal from water solutions and polyphenol removal from olive mill wastewater: Characterization, kinetics, adsorption performance, and mechanism. *Journal of Water Process Engineering*, **63**, 105432. https://doi.org/10.1016/j.jwpe.2024.105432

El Haimer, Y., Rich, A., Mountadar, S., Siniti, M., Tahiri, S., Mountadar, M. and Mangin, D. (2024) Enhancing phenolic compound recovery from olive oil mill wastewater through optimized cryoconcentration. *Journal of Crystal Growth*, **648**, 127904. https://doi.org/10.1016/j.jcrysgro.2024.127904

Faccioli, L. S., Klein, M. P., Borges, G. R., Dalanhol, C. S., Machado, I. C. K., Garavaglia, J. and Dal Bosco, S. M. (2021) Development of crackers with the addition of olive leaf flour (Olea europaea L.): Chemical and sensory characterization. *LWT,* **141**, 110848. https://doi.org/10.1016/j.lwt.2021.110848

Flamminii, F.; Gonzalez-Ortega, R.; Di Mattia, C. D.; Perito, M. A.; Mastrocola, D. and Pittia, P. (2021) Applications of compounds recovered from olive mill waste. In Food waste recovery (pp. 327-353). Academic Press. https://doi.org/10.1016/B978-0-12-820563-1.00006-8

Giusti, M. M., Gordillo, B. and González-Miret, M. L. (2024) Color Analysis. In: Ismail, B.P., Nielsen, S.S. (eds) Nielsen's Food Analysis. (Springer Cham), pp. 509-522 https://doi.org/10.1007/978-3-031-50643-7_31

Guo, J., Wang, F., Zhang, Z., Wu, D. and Bao, J. (2021) Characterization of gluten proteins in different parts of wheat grain and their effects on the textural quality of steamed bread. *Journal of Cereal Science*, **102**, 103368. https://doi.org/10.1016/j.jcs.2021.103368

Gupta, S., Shimray, C. A. and Venkateswara Rao, G. (2012) Influence of organic acids on rheological and bread-making characteristics of fortified wheat flour. *International journal of food sciences and nutrition*, **63**(4), 411-420. https://doi.org/10.3109/09637486.2011.631522

Gysel, M., Weingartner, E., Nyeki, S., Paulsen, D., Baltensperger, U., Galambos, I. and Kiss, G. (2004) Hygroscopic properties of water-soluble matter and humic-like organics in atmospheric fine aerosol. *Atmospheric Chemistry and Physics*, **4**(1), 35-50. https://doi.org/10.5194/acp-4-35-2004

Han, H. M. and Koh, B. K. (2011) Effect of phenolic acids on the rheological properties and proteins of hard wheat flour dough and bread. *Journal of the Science of Food and Agriculture*, **91**(13), 2495-2499. https://doi.org/10.1002/jsfa.4499

Jiang, G., Wu, Z., Ameer, K. and Song, C. (2021) Physicochemical, antioxidant, microstructural, and sensory characteristics of biscuits as affected by addition of onion residue. *Journal of Food Measurement and Characterization*, **15**(1), 817-825. https://doi.org/10.1007/s11694-020-00681-0

Karković Marković, A., Torić, J., Barbarić, M. and Jakobušić Brala, C. (2019) Hydroxytyrosol, tyrosol and derivatives and their potential effects on human health. *Molecules*, **24**(10), 2001. https://doi.org/10.3390/molecules24102001

Lalani, A. R., Rastegar-Pouyani, N., Askari, A., Tavajohi, S., Akbari, S. and Jafarzadeh, E. (2024) Food Additives, Benefits, and Side Effects: A Review Article. *Journal of Chemical Health Risks*, **14**(1).

Omar, S. H. (2010) Oleuropein in olive and its pharmacological effects. *Scientia pharmaceutica*, *78*(2), 133-154. https://doi.org/10.3797/scipharm.0912-18

Otero, P., Garcia-Oliveira, P., Carpena, M., Barral-Martinez, M., Chamorro, F., Echave, J., García-Pérez, P., Cao, H., Xiao, J., Simal-Gandara, J. and Prieto, M. A. (2021) Applications of by-products from the olive oil processing: Revalorization strategies based on target molecules and green extraction technologies. *Trends in Food Science & Technology*, **116**, 1084-1104. https://doi.org/10.1016/j.tifs.2021.09.007

Ou, J., Wang, M., Zheng, J. and Ou, S. (2019) Positive and negative effects of polyphenol incorporation in baked foods. *Food chemistry*, **284**, 90-99. https://doi.org/10.1016/j.foodchem.2019.01.096

Ouarouer, Y. (2019) Determination of kinetic parameters using Rancimat analysis. (Doctoral dissertation, Instituto Politecnico de Braganca (Portugal)) (p25).

Paciulli, M., Grimaldi, M., Rinaldi, M., Cavazza, A., Flamminii, F., Di Mattia, C., Gennari, M. and Chiavaro, E. (2023) Microencapsulated olive leaf extract enhances physicochemical stability of biscuits. *Future Foods*, **7**, 100209. https://doi.org/10.1016/j.fufo.2022.100209

Palmeri, R., Parafati, L., Trippa, D., Siracusa, L., Arena, E., Restuccia, C. and Fallico, B. (2019) Addition of olive leaf extract (OLE) for producing fortified fresh pasteurized milk with an extended shelf life. *Antioxidants*, **8**(8), 255. https://doi.org/10.3390/antiox8080255

Pampuri, A., Casson, A., Alamprese, C., Di Mattia, C. D., Piscopo, A., Difonzo, G., Conte, P., Paciulli, M., Tugnolo, A., Beghi, R., Casiraghi, E., Guidetti, R. and Giovenzana, V. (2021) Environmental impact of food preparations enriched with phenolic extracts from olive oil mill waste. *Foods*, **10**(5), 980. https://doi.org/10.3390/foods10050980

Passos, R. B. D., Bazzo, G. C., Almeida, A. D. R., Noronha, C. M. and Barreto, P. L. M. (2019) Evaluation of oxidative stability of mayonnaise containing poly ε-caprolactone nanoparticles loaded with thyme essential oil. *Brazilian Journal of Pharmaceutical Sciences*, **55**, e18177. https://doi.org/10.1590/s2175-97902019000118177

Penfield, M. and Campbell, A. M. (1990) Experimental food science, 3rd ed. (ed). Academic Press, Inc. London, P.33-34.

Pimpa, B., Kanjanasopa, D. and Boonlam, S. (2009) Effect of addition of antioxidants on the oxidative stability of refined bleached and deodorized palm olein. *Agriculture and Natural Resources*, **43**(2), 370-377. https://www.thaiscience.info/journals/Article/TKJN/10974287.pdf

Salazar Lopez, N. J., Loarca-Piña, G., Campos-Vega, R., Gaytán Martínez, M., Morales Sánchez, E., Esquerra-Brauer, J. M., Gonzalez-Aguilar, G. A. and Robles Sánchez, M. (2016) The extrusion process as an alternative for improving the biological potential of sorghum bran: Phenolic compounds and antiradical and anti-inflammatory capacity. *Evidence-Based Complementary and Alternative Medicine*, 2016(1), 8387975. https://doi.org/10.1155/2016/8387975

Silva, M. M. and Lidon, F. C. (2016[a]) Food preservatives-An overview on applications and side effects. *Emirates Journal of Food and Agriculture*, **28**(6), 366. doi: 10.9755/ejfa.2016-04-351

Silva, M. M. and Lidon, F. C. (2016[b]) An overview on applications and side effects of antioxidant food additives. *Emirates Journal of Food and Agriculture*, **28**(12), 823-832. doi: 10.9755/ejfa.2016-07-806

Smith, J. P., Daifas, D. P., El-Khoury, W., Koukoutsis, J. and El-Khoury, A. (2004) Shelf life and safety concerns of bakery products—a review. *Critical reviews in food science and nutrition*, **44**(1), 19-55.

https://doi.org/10.1080/10408690490263774

Stone, H., Bleibaum, R. N. and Thomas, H. A. (2020) Sensory evaluation practices. ٥nd Ed., Academic press. India.

Su, X., Wu, F., Zhang, Y., Yang, N., Chen, F., Jin, Z. and Xu, X. (2019) Effect of organic acids on bread quality improvement. *Food Chemistry*, **278**, 267-275. https://doi.org/10.1016/j.foodchem.2018.11.011

Subiria-Cueto, R., Coria-Oliveros, A. J., Wall-Medrano, A., Rodrigo-Garcia, J., González-Aguilar, G. A., MARTINEZ-RUIZ, N. D. R. and Alvarez-Parrilla, E. (2021) Antioxidant dietary fiber-based bakery products: a new alternative for using plant-by-products. *Food Science and Technology*, **42**, ctaAR57520. https://doi.org/10.1590/fst.57520

Uthayakumaran, S., Gras, P. W., Stoddard, F. L. and Bekes, F. (1999) Effect of varying protein content and glutenin-to-gliadin ratio on the functional properties of wheat dough. *Cereal chemistry*, **76**(3), 389-394. https://doi.org/10.1094/CCHEM.1999.76.3.389

Valenzuela-González, M., Rouzaud-Sández, O., Ledesma-Osuna, A. I., Astiazarán-García, H., Salazar-López, N. J., Vidal-Quintanar, R. L. and Robles-Sánchez, M. (2022) Bioaccessibility of phenolic compounds, antioxidant activity, and consumer acceptability of heat-treated quinoa cookies. *Food Science and Technology*, **42**, e43421. https://doi.org/10.1590/fst.43421

Zhao, B., Deng, J., Li, M., Li, H., Zhang, Y., Gong, H. and Chen, Z. (2020) Preparation and quality evaluation of potato steamed bread with wheat gluten. *Food Science & Nutrition*, **8**(8), 3989-3998. https://doi.org/10.1002/fsn3.1600