Egypt. J. Food. Sci. **Vol. 48,** No.1, pp. 27-40 (2020)

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

Utilization of Olive Pomace As A Source of Bioactive Compounds in Quality Improving of Toast Bread

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OLIVE oil pomace is produced as by-product with a large quantity during olive oil processing. It is a promising source for polyphenolic compounds and fibers which could be used in food industry. In this work proximate chemical analysis of olive pomace (two-phase olive oil extraction) was studied. Also, seven extracting solvents were tested in extracting the phenolic compounds from the olive pomace (OP). Total phenolic, flavonoids, and flavonols contents of the different extracts were determined. In addition to, the antioxidant activity of the phenolic extracts was investigated using 2, 2-diphenyl-1-picrihydrazyl (DPPH) to assess the extracting efficiency of solvents. The obtained data rivaled that protein, fat, ash and fiber contents of OP were 2.48, 2.33, 1.33 and 20.37% (FW), respectively. It is clear that the OP contains a large quantity of fibers and it had cellulose content about 40.7% of the fiber content. Furthermore, the total phenolic content was varied in the various extracts and ranged from 8.29 to 36.24 mg GAE g^{-1}. While, total flavonoids were ranged from 2.23 to 12.52 mg QE g^{-1}. Methanol and water (80:20) recorded the highest antioxidant activity with EC_{50} of 1.373µg/µg DPPH while, the acetone extract recorded the lowest antioxidant activity with EC_{50} of 8.052µg /µg DPPH. Toast bread was fortified with the cellulose isolated from OP at three replacement levels of 2, 4, and 6% and the results showed no significant differences between control sample and the sample fortified with 2% cellulose in most of sensory characteristics tested. Addition of pomace cellulose at replacement level of 2% enhanced the texture of the bead and was more acceptable than the control. The results concluded that olive pomace is a good source for dietary fibers and polyphenolic compound which could be used in the food industry.

Keywords: Olive pomace, Bioactive compounds and Toast bread.

Introduction

Olive oil showed very interested nutritional and sensorial properties due to its contents of unsaturated fatty acids and polyphenolic compounds which act as natural antioxidants to protect human body (Paz Aguilera et al., 2005). In addition to antioxidant activity of olive phenolic compounds, it was found to have antifungal activity (Winkelhausen et al., 2005). Extraction process of the olive oil production generates a considerable quantity of by-products which could cause serious environmental problems due to its very high organic loads (Roig et al., 2006). Those by-products found to consist of sugars, pectin, lipid, tannins and polyphenolic compounds. Utilization of it as a raw material for producing a high value compounds is particularly attractive way to reuse it (Niaomakis and Halvadakis, 2004).

Three different kinds of by-products are produced during the olive oil production depending on the production method used. (Fenandyez-Bolanos et al., 2006). The traditional

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Received:22/1/2020; accepted:27/2/2020
DOI: 10.21608/EJFS.2020.22871.1038

press method generates three phase and two by-products: olive oil about (20%), solid by-product (olive cake) around (30%) and waste water about (50%) of the olive fruits (Jerman Klen and Mozetic Vodopivec, 2012). On the other hand, the two-phase extraction system generates olive oil and one by-product which is a combination of liquid and solid. This by-product contains about 80% of the olive fruit which include pulp, skin, seeds and pieces of stones (Vlyssides et al., 2004). The by-product in this production method (olive pomace) differs in its chemical properties compared to olive cake from the traditional three-phase extraction method. It has higher moisture content ranged from 65-75% compared to 22-25% for the olive cake (Alburquerque et al., 2004). OP is the major output from the two-phase extraction method it is a good source for bioactive compounds which could be used in food manufacturing as well as pharmaceutical fields. Because of the high-water content of olive pomace, the concentration of water soluble salts and phenolic compounds in it were higher than that of the olive cake produced by the three-phase extraction system (Dermeche et al., 2013). Those bioactive compounds could have new application in foods and cosmetic industries (Rodrigues et al., 2017).

The quantity of olive pomace generated during production of olive oil is ranged from 2.75 to 4 tons for each ton of oil depending on fruit quality, and extraction technology (Rorja et al., 2006 and Conterno et al., 2017). OP was found to have a considerable amount of polyphenoilc compound. It was reported that about 89% of olive fruit phenolic compounds remains in olive pomace for which the functional properties of olive oil and olive pomace are related (Ghanbari et al., 2012). Olive pomace phenolic profile has been studied and found to be fared according to many factors including, fruit ripening, climatic condition, cultivar, origin, and the extraction system (Obied et al., 2008). Some authors found oleuropein and oleuropein derivatives as major compounds (Cioffi et al., 2010). Other researchers reported different major compounds, namely hydroxytyrosol as the main phenolic compounds in olive pomace. (Alu'datt et al., 2010; Rubio-Senen et al., 2012 and Nunes et al., 2018). The major components in the olive pomace are dietary fiber (including cellulose, hemicellulose, lignin and pectin), protein, lipids, pigment, and polyphenolic compounds (Di Gioia et al., 2002). The inclusions of fibre in the food's formulation will inevitably bring about certain changes to the product. Utilization of OP to obtain natural ingredients like phenolic compounds and dietary fibers to develop new food products is a proms subject (Nunes et al., 2018).

Cellulose is insoluble in cold or hot water and in hot dilute acids or alkalis. (Singanuson et al., 2014). Powdered cellulose used in many food applications, essentially in functional bread, to increase fibre content and to decrease nutritional value of the bread. Addition of cellulose to the bread formulation, results in many changes in the quality properties of the bread (Poran et al., 2008). It is increasing the viscosity of the liquid mixture of flour because its insolubility in water. This leads to form a stronger gas holding structure. Thus, the air bubbles will remain in small sizes and will not float on the surface of the liquid, which results in the product having accurate air bubbles, larger foam size and better stability (Wongsonsarim et al., 2001).. cellulose powder was announced to use as a bulking agent in drink products, meat products, salads, and especially baked and cereal products.

In this current work, various extracting solvents were tested for extracting the phenolic compounds from tow-phase extraction olive pomace. Total phenolic, flavonoids, flavonols contents were also determined and identified using HPLC/DAD- MS. The antioxidant activity of the different phenolic extracts was investigated using DPPH method. Cellulose was isolated from the OP as functional food ingredient and used at different concentrations in toast bread formulation to investigate its effects in the quality proprieties of the produced toast.

Materials and Methods

Materials and reagents

Olive pomace (4 kg) from tow-phase extraction olive oil unit was acquired from olive production factory in Fayoum, Egypt. The sample was homogenized and stored at -20º C until analysis. All chemicals used were analytical grade and purchased from sigma-Aldrich, Berlin, Germany

Proximate chemical analysis

Moisture content was determined using oven at 50°C under vacuum for 48 hr and the dried sample was ground to fine powder for using in farther analysis. Fat, total protein, ash, and fiber of OP and toast bread were determined according to (AOAC, 2012). Total protein content was calculated using 6.25 as the nitrogen conversion factor (Nunes et al., 2018). Available carbohydrates were calculated by difference.

Extraction of phenolic compounds from olive pomace

The dried Olive pomace was first extracted which n-hexane in at ratio (1: 4 W/V) three times successively to remove the residuals of fats and pigments. Then, the polyphenols were extracted from the OP using varies extracting solvents including acetone, ethanol, ethanol and water (50:50), methanol, methanol and water (80: 20), ethyl acetate, and water at solvent to pomace ratio (10: 1). The extraction process was cured out in a shaker at room temperature for 24 hr followed by filtration. The residues were re-extracted under the seam conditions. The total extracts were filtered (0.45 µm) and the solvents were evaporated at 35°C. in a Speed Vacuum Concentrator, SPD111V 230 (Thermo scientific, USA). The yield was calculated and stored in dark at -20 °C until used (Alu'datt et al., 2010).

Determination of total phenolic content

Total phenolic content of the extracts was determined spectro-photometrcally with Folin–Ciocalteu assay (Vergani et al., 2014). A 20 µL aliquot of extracts solutions were mixed with 100 µL of Folin–Ciocalteu's reagent followed by 1.586 mL of distilled water and followed by 300 µL of 20% Na_2CO_3 solution. The obtained mixtures were incubated in a shaking incubator at 40 °C for 30 min and the absorbance was measured at 765 nm. The results were expressed as mg gallic acid equivalent (GAE) using the following liner equation based on gallic acid calibration curve.

$$Y = 0.0248x + 0.237 \ (R^2 = 0.997)$$

Where A is the absorbance and C is the concentration (mg GAE g−1 dry weight (DW))

Determination of total flavonoids and flavonols

Total flavonoids content was determined as described by Mohdaly et al. (2009). Using $AlCl_3$ ethanolic solution and the absorbance was measured at 420 nm. Total flavonoids content calculated and expressed as quercetin equivalent (QE) using the following equation based on the calibration curve:

$$Y = 0.0035x + 0.0258 \ ((R^2 = 0.9929)$$

Where Y is the concentration (mg QE/100 g extract) and X is the absorbance and Total flavonols content was determined according to the method described by Kumaran and Joel (2007). 2 mL of 20 g/L^{-1} $AlCl_3$ ethanolic solution and 3 mL of 50 g/L^{-1} sodium acetate solution were added to 2 mL of extract solution. The absorption was recorded after 2.5 hr at 440 nm. Total flavonols content expressed as QE was calculated using the quercetin calibration curve:

Antioxidant activity of olive pomace extracts

The antioxidant capacity pomace extracts were determined using 2,2-diphenyl-1-picrihydrazyl ($DPPH^{\bullet}$) assay according the method of Brand-Williams et al., (1995) with some modifications. Extracts and synthetic antioxidants (BHA, BHT and TBHQ in ethanol) solutions of different concentrations (100µL of each) were vortexed for 30 s with 1.8 mL of DPPH solution and left to react for 30 min, after which the absorbency of the remaining DPPH was recorded at 515 nm. A control sample was prepared at the same conditions without extract. The measurements were performed in triplicate. Scavenging activity was calculated as follows:

$$\text{scavenging (\%)} = [(\text{A. control} - \text{A. sample}) / \text{A. control}] \times 100$$

Where : A is the absorbance at 515 nm.

Antiradical Efficiency = $1/EC_{50}$

EC_{50} is extraction concentration providing 50% inhibition of DPPH.

High performance liquid chromatography (HPLC/DAD)

HPLC was performed in LC10 AD HPLC eluent pump (Shimadzo, Kyoto, Japan), DAD SPDM10 AVP, UV-Vis SPD 10AVP detectors (Shimadzo, Kyoto, Japan). phenolic compounds were separated on a C18 analytical column, 250 .46 mm i.d. (Shimadzo, Kyoto, Japan) packed with Luna C18 stationary phase, particle size 3µm and connected to C18 precolumn. The time of HPLC run was over 40 min. UV-Vis detector was operating at 290 nm wavelength. Phenolic compounds were identified by comparison of chromatographic retention times and area of the peak in the extract with that of the standard phenolic acids and reference compounds using Mass Lynx 4.0 software (Micromas UR Ltd., UK) and the available literature data (Gheldof et al., 2002).

Determination of cellulose content

This was achieved by delignification of the sample with chlorous acid followed by elimination of hemicellulose by treatment of the resulting holocellulose with 10 % NaOH at 80°C for 3 hours as reported by Chen et al. (1988) as follows: The lignocelluloses material was first subjected to Soxhlet extraction with a mixture of methanol and benzene (1:1) for 8 hr lignin was then eliminated

treating 5.0 grams (oven dry) of olive cake with sodium chlorite solution (sodium chloride 1.5 gram in 160 mls. of water containing 10 drops of glacial acetic acid). The mixture was left in water bath at 70°C for one hour then after, ten drops of glacial acetic acid and 1.5 gram of sodium chlorite were again added and this was repeated twice. After filtration the residual material was washed successively with water and ethyl alcohol to give the holocellulose. For the removal of hemicellulose, the obtained holocellulose was treated 10 % NaOH solution (1:20 W/V) and heated at 80°C in a water bath for 1.5 – 3.0 hour. After filtration, the residue was washed with water, ethanol and ether and then dried at 105°C to constant weight.

Determination of hemicellulose content

Hemicellulose content was estimated, gravimetrically, after extraction, as reported by Chen et al. (1988) 10 grams of olive cake with 14 % NaOH (100ml) at 90ºC for 3 hr, and precipitated with HCl at PH 5. The precipitated hemicellulose was separated by centrifugation, washed with ethanol, then dried and weighted.

Determination of lignin content

Lignin content in the investigated materials was also determined according to the method described by Fahmi (1984) as follows: One gram olive cake extracted using a mixture of methanol and benzene (1:1). In a wide mouthed bottle of 150 ml capacity, 50 ml of 38% pure HCL (not less than 38%) was added. The mixture was left for two min, and then 50 ml of concentrated sulfuric acid was added. After shaking for one hour the mixture was left for 24 hr at 22˚C. The content was poured in a beaker of one-liter capacity. The bottle was washed with 415 ml of distilled water and filtered. The obtained was dried at 105˚C for 3 hr and weighted.

Cellulose isolation

The isolation of cellulose was cured out using the methods described by Brendel et al. (2000) as follows: 500 gram of dried olive cake was extracted with aqueous acetic acid 80% (w/w) and nitric acid 70% (w/w). The residue was then thoroughly washed with distilled water and ethanol 95% to remove the nitric acid and extraction breakdown products. The residue was then dried in an oven at 60 ºC for 16 hr and was labeled as cellulose.

Determination of dough rheological properties

The effect of powdered cellulose on rheological properties of dough during mixing was determined by the Brabender Farinograph, following the AACC Approved methods (54 – 21, 1983) and the Brabender Extensigraph following the AACC Approved methods (54 – 10, 1983).

Bread making

The Straight Dough (Bulk Fermentation) method was used for bread making as described by Pourabedin et al. (2017), the amount of added water was calculated from Table 6. The control sample was consisted of 100 g flour, 1.5 g salt, 1g yeast. Three treatments ware prepared with three different replacement levels 0, 2, 4 and 6 % of the flour. Dough pieces (100 ± 0.1 g) proofed for 90 minutes at 30 ˚C and 90 % relative humidity. Bread was baked at 250˚C for 25 min.

Sensory evaluation

Toast bread containing different cellulose powders and control bread were sensory tested in Food Technology Department, Faculty of Agriculture, Kaferelsheikh University, Egypt. For their color, appearance, odor, texture, taste and overall acceptability on a 1 to 10 hedonic scale as described by Meilgaard et al. (2007).

Statistical analysis

Statistical analysis of the data obtained from the study was performed using the SPSS v statistical program SPSS v. 16 for windows (SPSS, Chicago, II., USA) The ANOVA method was used to analyze the results. Multiple range tests from Duncan were used at a level of 5% of significance for comparison of means. All trials were conducted in triplicate.

Results and Discussion

Proximate chemical composition of olive pomace

The main values obtained for the proximate composition of OP are presented in Table 1. The results showed that the sample presented a moisture content of 64.58% (FW) and protein content of 2.48%. The data rivaled that ash and fiber content of OP was 1.33 and 20.37% (FW) respectively. The total lipid of the analysed sample was 2.33g/100g (FW) which could be used in food and cosmetics industry (Rodrigues et al., 2017). Di Giovacchino and Prezioso, (2006) reported that ash content of OP ranged from 1.42 to 4% (FW). It is clear

that the OP contains a large quantity of fibers and it had cellulose content about 40.7% of the fiber content. Several studies have focused on isolation of dietary fibers from olive pomace and reported cellulose, hemicellulos and lignin as the main carbohydrates in olive by-products (Jimenez et al., 1994). In another study, Vlyssides et al., (1998) found that OP contains cellulose, hemicellulos and lignin of 14.54, 6.68, and 8.54% (DW) respectively. These compounds could be used in many food products as gelling agents, fiber source and fat substitute. It was found that the proximate composition of OP is influences by many factors including ripening stage, cultivar, and agriculture practices (Portarena et al., 2017). Our results agreed with that stated by Roselló-Soto et al. (2015) and Nunes et al. (2018).

TABLE 1. chemical composition of olive pomace.

Olive pomace	g /100g (FW)
Moisture content	64.58± 2.51
Total lipids	2.33± 0.06
Protein	2.48±0.04
Ash	1.13±0.02
Total carbohydrates	29.48± 2.41
Cud fiber	20.37± 1.65
Cellulose*	40.77 ± 2.44
Hemicelluloses*	33.63±2.28
Lignin*	19.50± 1.79

*Calculated as a percentage from pomace fiber (% on dry weight basis)

Extraction of phenolic compounds from olive pomace by different solvents

The majority of polyphenolic compounds (98%) remain in the olive pomace and a small fraction passes into the oil about (2%). (Chanioti and Tzia, 2017). To extract the phenolic compounds from OP, suitable solvents must be used according to their extraction efficiency and according to the uses of the compounds to be extracted. The effect of various solvents was assessed to determine the most appropriate one for yield, total phenoilc, flavonoids, and flavonol content of the extracts obtained. Polyphenolic yields were found to increase with increasing the polarity of the solvents. The extract yield was varied from 141.27 to 66.92 g / kg -1 (DW) (Table 2). The obtained results proved that using methanol and water showed highest yield , while acetone scored the minimal polyphenol production (66.92 g / kg DW).

Referring to polyphenols from different extracts, the results showed that the polyphenols content varied from 36.24 to 8.29 mg/g^{-1}. Methanolic extract recorded the highest total polyphenolic content higher than the other solvents While, hand, acetone extract presented the less polyphenolic contents. This because of the high polarity of methanol. The obtained findings similar to those obtained from other researchers who used several solvents to extract phenolic compounds from olive and olive pomac. (Cioffi et al, 2010, Lafka et al, 2011, Vergani et al, 2016, Araújo , 2015 and Albahari et al., 2018). Cedola et al. (2019) found that dry olive paste had phenols content, equal to 45.09 mg GAE/g dw, and high amount of flavonoids (36.11 mg QE/g dw).

TABLE 2. The influence of different solvents on yield and total phenolic content of olive pomace.

Solvents	Extract yield (g/ kg^{-1} DW)	Total phenolic (mg GAE g^{-1} DW)	EC_{50} µg/µg DPPH	Antiradical efficiency
Methanol	115.85±0.22	24.64±0.05	2.337	0.428
Ethanol	98.68±0.21	18.22±0.04	4.416	0.226
Methanol: water (80:20)	141.27±0.28	36.24±0.10	1.373	0.728
Ethanol: water (50:50)	118.61±0.34	26.22±0.08	1.733	0.577
Ethyl acetate	80.51±0.17	9.04±0.02	5.821	0.172
Acetone	66.92±0.13	8.29±0.03	8.052	0.124
Water	94.58±0.19	19.50±0.07	2.795	0.358

Total flavonoids and flavonols content of olive pomace extracts

Total flavonoids and flavonols content of olive pomace extracts were determined to identify the quality and the structure of the OP phenolic compounds. Because of the antioxidant efficiency dose not correlate with the quantity of the phenolic compounds in all cases. The results are presented in Fig. 1. The results indicated that flavonoids and flavonols content were varied with the different extracts. Total flavonoid contents ranged from 12.52 mg/g^{-1} (DW) for methanol + water extract to 2.23 mg/g^{-1} (DW) for acetone extract. Regarding to the flavonols contents, methanol + water and ethanol+ water recorded the highest content with values of 6.73 and 5.72 mg/g^{-1} (DW) respectively. On the other hand, aceton and ethyl acetate recorded the lowest flavonoids and flavonol contents of the olive pomace.

Identification of polyphenolic compounds from methanol: water extract of olive pomace

The phenolic compounds and derivatives of olive pomace methnolic extract in this study were fractionated an identified by HPLC/DAD. The protective chromatogram obtained at 290nm along with the corresponding retention time, peaks number and beak relative area are presented in Fig. 2 and Table 3. The prime polyphenolic found in the studied extract are phenolic-acids, phenolic-alcohols, secoiridoid derivatives, and flavonoids. The major phenolic acids were galic, vanillic, caffeic ,and ρ- coumaric acids. Caffeic acid showed the highest concentration, while galic acid and cinnamic acid recorded the lowest concentration in the methanolic pomace extract. Many factors were found to affect the phenolic compounds concentrations including the cultivar, agronomic, geographic origin of olive, olive tree irrigation, and technological conditions of oil production (Servili and Montendoro, 2002). These results agree with those reported by Vincenzo (2016) and Cioffi et al. (2010).

The chromatogram of olive pomace brings to light the presence of hydroxytyrosol and tyrosol as phenolic alcohols and oleuropein, ligstroside aglycone, and oleuropein aglycone as secoiridoid derivatives. Hdroxytyrosol have been revealed to be one of the most interesting phenolic compounds in the olive pomace, because of its strong antioxidant activity, anti-inflammatory and antimicrobial properties (Robles-Almazan, 2018). Moreover, this compound affects the quality properties (sensory and chemical) of food products was explored (Nunes et al., 20180). Oleuropein and oleuropein aglycone were found in olive oil and olive pomace (Fernendez-Bolanes et al., 2006, Goldsmith et al., 2014, Leouifoudi et al., 2014. and Sicari, 2016). Oleuropein also works as natural anti-oxidant in the body (Visioli et al., 2006). The results showed the presence Rutin and luteolin as flavonoids derivatives in the olive pomace. According to the literature data, these compounds were detected in olive oil and olive by-products (Dermeche et al., 2013).

Antioxidant activity of olive pomace extracts

Because of the undesirable effects of the synthetic antioxidants on human health and enzymes systems, the natural antioxidants can replace the synthetic one (Monica et al., 2007 and Baydar et al., 2007). The results of scavenging effects of the OP extracts are summarized in Fig. 3. It could be notice that inhibition ratio is increases by increasing the concentrations of extracts for all tested extracts. when low concentration 50µg/mL was used, the remaining DPPH hanged from 93.63% for acetone extract to 60.38% for methanol+water extract. Meanwhile, when the concentration was increased to 300 µg/mL the remaining of DPPH was decreased to 64.72, 8.38, and 6.65% for acetone, ethanol +water, and methanol+water extracts respectively. These results may be due to the elevated polyphenolic content in methanol and ethanol extracts. Our findings are compatible with that stated by (Silva et al, 2006, Obied et al*.,* 2007, and Leouifoudi et al., 2014).

The results in the same figure illustrate the scavenging activity of the OP extracts at different concentrations compared to the synthetic antioxidants (BHA, BHT, and BHTQ). Scavenging activity of OP was found to be concentration dependent. The results showed that methanol+water and ethanol+water had high scavenging activity as strong as BHA and BHT and recorded EC_{50} values of 1.373 and 1.728µg/ µg DPPH and antiradical efficiency values of 0.728 and 0.577, respectively. The results also proved that acetone extract showed the lowest scavenging antioxidant activity with EC_{50} value of 8.052µg/µg DPPH. The strong scavenging activity could be related to the high concentration of the antioxidant compounds in OP such as hydroxytyrosol, oleuropein, and oleuropein aglycon (Suarez et al., 2009 and Leouifoudi et al., 2014).

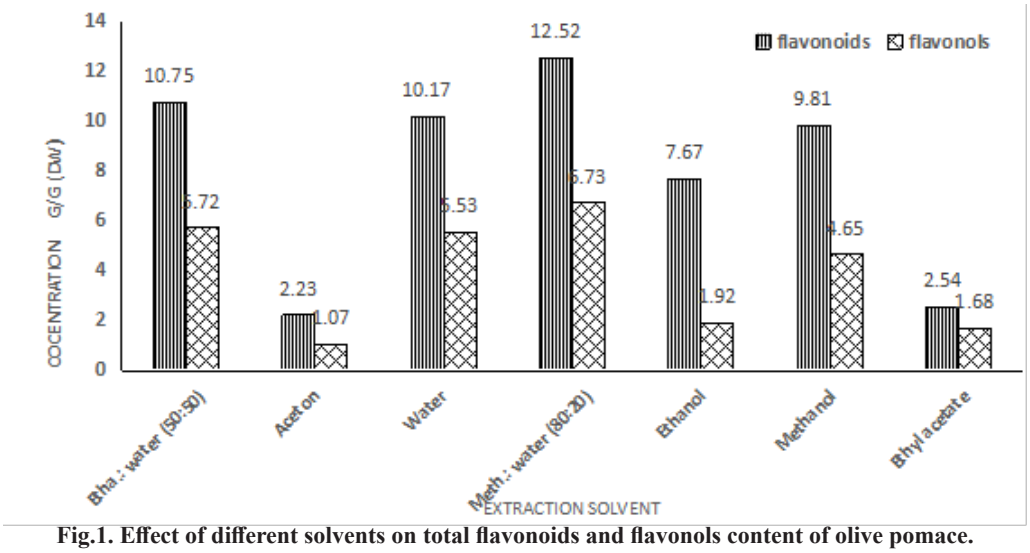

Fig.1. Effect of different solvents on total flavonoids and flavonols content of olive pomace.

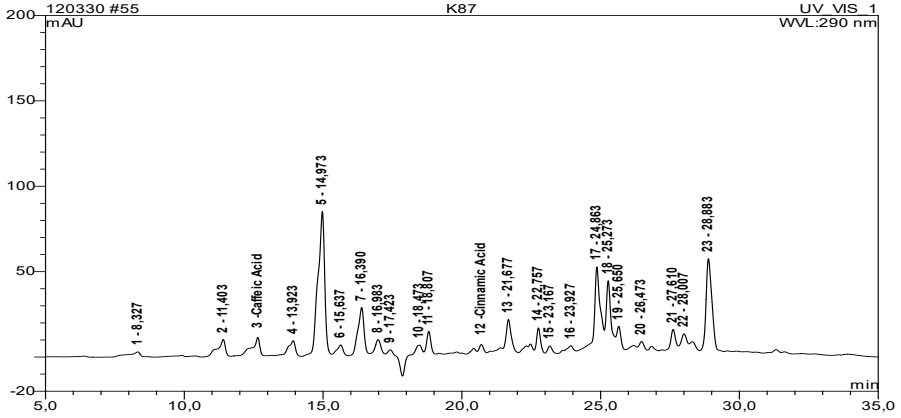

Fig.2. HPLC–DAD chromatogram of olive pomace methanolic extract.

TABLE 3. Phenolic components of olive pomace methanolic extract.

No.	R. T. Min.	Peak Name	Height mAU	Area mAU*min	Relative Area %
1	8.33	Gallic acid	2.893	1.390	1.22
2	11.4	Hydroxytyrosol	10.090	3.342	2.93
3	12.65	Caffeic acid	10.873	3.706	3.25
4	13.92	Cumaric acid	9.062	2.684	2.36
5	14.97	Tyrosol	84.547	23.963	21.03
6	15.64	Vanillic acid	6.272	1.652	1.45
7	16.39	Oleuropein	27.784	7.064	6.20
8	16.98	NA	8.605	1.999	1.75
9	17.42	Ligstroside aglycone	6.360	2.967	2.60
10	18.47	NA	14.712	8.113	7.12
11	18.81	oleuropein aglycone	20.897	9.379	8.23
12	20.7	Cinnamic acid	4.391	0.691	0.61
13	21.68	NA	17.878	3.555	3.12
14	22.76	Ferulic acid	13.727	2.074	1.82
15	23.17	NA	4.207	0.748	0.66
16	23.93	NA	2.932	0.552	0.48
17	24.86	Oleuropein glucoside	48.869	12.416	10.90
18	25.27	Luteolin	40.621	8.189	7.19
19	25.65	Luteolin-7-glucoside	13.812	2.484	2.18
20	26.61	NA	4.130	0.688	0.60
21	27.61	Rutin	10.918	1.886	1.65
22	28.01	NA	6.671	1.217	1.07
23	28.88	NA	53.992	13.190	11.58

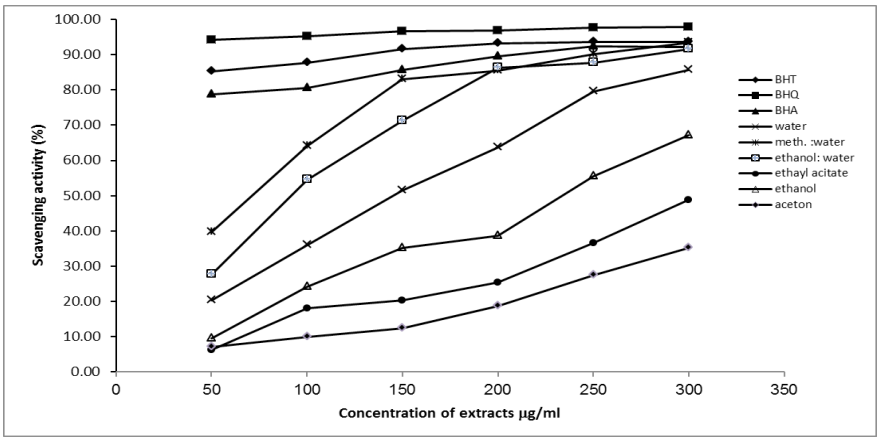

Fig. 3. Scavenging activity of olive pomace at assorted concentration against DPPH radical and comparing with synthetic antioxidants .

Sensory evaluation of toast bread samples fortified with olive pomace cellulose

Cellulose is one of the main olive cell wall polysaccharides recovered from olive pomace (Catanakis et al., 2010). In food industry, the valorization of native dietary fibers appears to be to be interesting fields, it is now well-known to have role in prevention of several diseases such as cancer (Rodríguez et al., 2006). There are less publications on the utilization of olive pomace in foods especially its effects on the rheological properties of the dough when used as a substitute for wheat flour in bread making. In this part of the study, isolated cellulose from olive pomace was used in fortification of toast bread and its effects on the rheological and sensory properties of the bread was investigated.Results of sensory evaluation of produced bread with different added levels of OP cellulose are given in Table 4. It indicated no significant differences between the control and the sample fortified by 2% cellulose in all the tested parameters. The results showed that increasing the fortification ratio to 4% led to no significant differences ($p < 0.05$) between the control and the sample in color, taste and texture and significant differences in, odour, appearance and overall acceptability.

The bread with 2% substitution level was superior in all sensory characteristics evaluated compared to other replacement ratios. Moreover, it recorded higher scores in texture, appearance and overall acceptability than the control sample. When 6% of the flour were replaced with cellulose,significant drops in the values of all the sensory characteristics were observed in the resulting bread. Our results are similar to that presented by Cecchi, et al. (2019) for pasta, bread, and granola bar fortified with olive pomace. Cedola et al. (2019) found that addition of dry olive paste flour to the bread also slightly interfered with the network formation, thus influencing the final bread bubbles that were considered more acceptable in the control samples than in the enriched bread. The Sensory evaluation of cellulose-enriched baked rolls indicated that addition of cellulose by up to 1% was identical to the control rolls. It was also found that increasing the addition levels of cellulose significantly reduced the crus, taste, porosity and color of the produced rolls (Lauková et al. (2017). Likewise, Gómez et al. (2003) mentioned that it is possible to add dietary fibers to the flour while making bread by up to 2% without. Ang and Miller (1989) used cellulose powder in the cake manufacture with different addition ratios and measured the texture of the cake. They found that the low levels of cellulose addition led to the cake being produced more harder texture than those with a high level of cellulose. This can be because long fibers contribute to a soft cake. They also noticed that these ratios from adding up to 4% improve the overall appearance and reduce moisture loss from the surface cracking as well as extending the shelf life of the cake (Kamel and Stauffeer, 1993 and Prakongpan et al, 2002).

Quality characteristics of toast bread

Regarding to the effect of adding cellulose in the bread formula in the chemical composition of the produced bread, the results in Table 5 showed that moisture content of the bead was increased with increasing the fortification level. There were no significant differences between

the control sample and the sample fortified with 2% olive cellulose. As prospective, it was noted that the bread samples to which cellulose was added contained higher fibers than those made without adding cellulose. Besides, the results also demonstrated no significantly differences among the control and the bread samples with 2% and 4% added olive cellulose powder in protein, lipid and ash contents. Our results were similar to that obtained by Lin et al. (2017). For olive pomace based high fiber biscuit. Sodchit et al. (2013) added banana peel cellulose powder to butter cake at three levels and found that addition cellulose to the cake improved the fiber content of the product. They also observed that sample with 1.5 % added cellulose recorded no significant differences compared with the control in protein, moisture, total lipid and ash contents.

Effect of addition of olive pomace cellulose on the rheological properties of the dough

Farinograph characteristics

It was found that adding dietary fibers to the bread improves the nutritional value, but at the same time it may lead to a change in some of the rheological properties of the dough, and this may lead to an effect on the quality of the bread and the sensory properties of bread (Rosell et al., 2005). Farinographic properties of doughs with several levels of olive pomace cellulose are given in Table (6). Water is an important component of baking dough because it takes part in hydration of gluten, thus ensuring that the dough maintained carbon dioxide. Also, the ability of dough to absorb water (WA) is of great importance in bread industry. The water absorption of dough with 2% olive pomace cellulose substitution was 54.5% and it is higher than that of control dough. When the olive

Extensograph properties

According to the results of extensographic properties of doughs with added olive pomace cellulose (Table 6), the controls sample recorded the highest extensibility while the sample substituted with 6 % cellulose showed the lowest extensibility value. Thus, it can be observed that addition of olive pomace cellulose caused the extensibility of the dough samples to decrease. Similar findings were reported by Koca and Anil (2007) for replacement of wheat flour with flaxseed flour in bread. Likewise, Poran et al. (2008) found that addition of cellulose to the wheat flour reduced the extensibility of dough. This finding could be attributed to the protein content decreases when flour is substituted by powdered cellulose and as a result, the dough loses portion of its expandability property. A consequence, the dough loses part of its extensibility property. Also, the dough demand for water increased when fibre was added and these led to dilution of gluten and reduce extensibility of the dough. The resistances to extension of dough samples are presented in Table 6. The results indicated that there was a highly significant increase in the resistance of dough, when the flour was substituted with powdered cellulose. The resistance of the dough increased from for the control to for 6% cellulose flour sample. Such increment in the resistance could be due to the interaction between the olive cellulose and gluten in wheat flour. Many studies declared negative effect of adding insoluble fiber on the formation of gluten network (Pourabedin et al., 2017, and Ahmed et al., 2013). The energy value of dough with 2% olive pomace cellulose substitution was analogous to the control. However, the energy values of doughs made with 4% and 6% olive pomace cellulose were significantly lower than that of control dough.

TABLE 4. Sensory evaluation of toast bread fortified with olive pomace cellulose.

Samples	Sensory characteristics					
	Color	Taste	Odour	Texture	Appearance	Overall acceptability
Control	9.20 ±0.15^{a}	9.23 ±0.37^{a}	9.04 ±0.34^{a}	8.20 ±0.39^{ab}	9.11 ±0.22^{a}	8.23 ±0.27^{a}
2 % OPC*	8.8 ±0.38^{a}	8.92 ±0.54^{a}	8.91±0.12^{a}	8.90 ±0.18^{a}	8.95 ±0.32^{a}	8.53 ±0.44^{a}
4 % OPC	8.75 ±0.29^{a}	8.85 ±0.42^{a}	8.17 ±0.19^{b}	8.10 ±0.32^{b}	8.38 ±0.17^{b}	8.16 ±0.14^{b}
6 % OPC	7.94 ±0.41^{b}	6.81±0.22^{b}	6.90 ±0.71^{c}	7.43 ±0.54^{c}	6.82 ±0.27^{c}	7.14 ±0.19^{c}

OPC= olive pomace cellulose

TABLE 5. Chemical composition of toast bread fortified with 2, 4 and 6% olive pomace cellulose.

Samples	Chemical composition (%)					
	Moisture	Protein	Ash	Total lipids	Fiber	Carbohydrates
Control	11.20±0.23^{b}	10.95±0.33^{b}	1.79±0.14^{a}	3.80±0.43^{a}	3.22±0.32^{d}	80.66±1.40^{a}
2 % OPC	11.44±0.40^{b}	10.71±0.27^{b}	1.78±0.19^{a}	3.79 ±0.23^{a}	3.90±0.41^{c}	79.92±1.55^{a}
4 % OPC	11.98±0.64^{a}	10.22±0.29^{ab}	1.77±0.09^{a}	3.78±0.28^{a}	4.38±0.50^{b}	79.55±1.70^{a}
6 % OPC	12.09±0.92^{a}	9.69±0.74^{a}	1.75±0.11^{a}	3.76±0.33^{a}	5.14±0.40^{a}	79.26±1.60^{a}

OPC= olive pomace cellulose

TABLE 6. Effect of olive pomace cellulose addition on Farinogragh and Extensograph parameters of wheat flour (82% extraction).

parameters / Samples	Farinogragh parameters				
	Water absorption %	Arrival time (min)	Developing time (min)	Stability time (min)	Weakening value (BU)
Control	53.6^{c}	0.5^{c}	1.0^{c}	4.50^{a}	70^{c}
2%	54.5^{b}	1.5^{b}	2.0^{b}	3.52^{b}	80^{b}
4%	56.9^{b}	1.5^{b}	2.25^{b}	3.51^{b}	80.5^{b}
6%	66.8^{a}	2.2^{a}	3.1^{a}	3.04^{c}	90.7^{a}
	Extensograph parameters				
	Extensibility (mm)	Resistance (BU)	proportional number	Energy (cm^{2})	
Control	125	445	3.65	63	
2%	110	462	4.21	58	
4%	101	468	4.63	48	
6%	95	484	5.09	40	

Conclusion

This research confirmed that the quantity of polyphenolic compoundst in the olive pomace were ranged between 36.24 to 8.29 mg/g^{-1} DW depending on the extraction solvent used. The major polyphenolic compounds in the olive pomace were tyrosol, hydroxytyrosol, oleuropein, and oleuropein aglycon, luteolin and rutin. The results showed that olive pomace methanolic extracts had high scavenging activity as strong as BHA and BHT which could be related to the high concentration of the antioxidant compounds in OP such as hydroxytyrosol, oleuropein, and oleuropein aglycon. Adding olive pomace cellulose powder to wheat flour led to significant alters in farinograph parameters. Addition of 2% cellulose powder enhanced the texture and the acceptability of the toast bread compared with the control sample, . It can be concluded that olive pomace cellulose could be used as wheat replacer in toast making at the level of 2% with no impairment on quality characteristics of the bread.

Acknowledgment

This study was financial propped by Cultural Affairs and Missions Sector, Egypt and University of Applied Science Weihenstephan-Triesdorf, Weidenbach, Germany.

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الاستفادة من كسب الزيتون كمصدر للمركبات النشطة في تحسين جودة الخبز المحمص

ينتج كسب الزيتون بكميات كبيره كمنتج ثانوى اثناء انتاج زيت الزيتون. فى هذا الدراسة تمت دراسة التركيب الكيميائى لكسب الزيتون الناتج من استخلاص زيت الزيتون بطريقة two phase extraction وتم استخلاص المواد الفينوليه من الكسب الناتج باستخدام سبعة مذيبات مختلفة وتم تقدير الفينولات والفلافونيدات والفلافونات الكلية وكذالك تقدير النشاط المضاد للاكسدة باستخدام مركب 2,2-diphenyl-1-picrihydrazyl (DPPH). في المستخلصات الناتجة.

أوضحت نتائج الدراسة ان كمية الفينولات الكلية تختلف باختلاف المذيب المستخدم في الاستخلاص حيث تراوحت بين ٨,٢٩ الى ٣٦,٤٢مجم/جم كسب جاف بينما تراوحت كمية الفلافونيدات ما بين ٢,٢٣ الى ١١,٥٢مجم/جم كسب جاف. واظهرت النتائج ان استخدام الميثانول والماء (٨٠: ٢٠) كمذيب استخلاص اعطى اعلى كمية من المواد الفينولية وان المستخلص الناتج باستخدام هذا المذيب اعطى اعلى نشاط مضاد للاكسدة مقارنة بالمذيبات الأخرى, واظهر استخدام الاسيتون كمذيب استخلاص اقل كمية من الفينولات الكليه واقل نشاط مضاد للاكسدة.

تم استخدام السليلوز المفصول من كسب الزيتون في انتاج خبز التوست بثلاث نسب استبدال ٢و ٤و ٦٪ من الدقيق لدراسة تاثير الاستبدال على الخواص الكيميائية والحسية للخبز التاتج. وبينت النتائج ان جميع نسب الاستبدال أدت الى زيادة نسبة الالياف في الخبز الناتج وانخفاض طفيف في نسبة الدهون والبروتين خصوصا عن استخدام ٢٪ استبدال. أظهرت نتائج التقييم الحسى عدم وجود فروق معنويه بين العينة الكنترول والعينه المنتجه باستخدام نسبة استبدال ٢٪ في وسجلت تحسن في صفة القوام والقبول العام مقارنة بالكنترول. يمكن من النتائج المتحصل عليها استخدام كسب الزيتون كمصدر للمواد الفينولية كمضادات اكسدة وكذلك كمصدر للالياف الغذائية التيمكن استخدامها في المنتجات الغذائية المختلفة.