Jordan Journal of Agricultural Sciences, Volume **19**, No.**4 2023**
DOI: https://doi.org/10.35516/jjas.v19i4.432

The Antimicrobial and Antioxidation Activities of Olive Pomace Extract in Pasteurized White Cheese

Hadjer M. Ferguous [1] **, *Ghadeer F. Mehyar**[1] *and Salam A. Ibrahim*** [2]

[1] Department of Nutrition and Food Technology, School of Agriculture, The University of Jordan, Amman11942, Jordan

[2] 171 Carver Hall, Food Microbiology and Biotechnology Laboratory, College of Agriculture and Environmental Sciences, North Carolina A & T State University, Greensboro, NC 2711-1064

Received on 20/9/2022 and Accepted for Publication on 26/2/2023

ABSTRACT

Olive pomace from an olive mill was collected in December 2021. Proximate analysis revealed that the major constituents of the pomace were fiber (60.85 %) and fat (11.91%). Extraction of the olive pomace by methanol provided a higher yield, phenolic content, and free radical scavenging ability than the ethanol extract, but the latter was used due to its lower toxicity level as the extract is used in foods. The extract contained high concentrations of phenolic compounds, particularly syringic, p-coumaric, vanillin, and caffeic acid which accounted for ~80% of the total phenolic content. When the olive pomace extract (OPE) was used (0.08%) in pasteurized white cheese (PWC), the counts of mesophilic aerobes were significantly reduced to 3.62 log10 CFU/g after 30d of storage at 5°C compared to the control (4.04 log10CFU/g) after the same storage period. The corresponding values for LAB were 3.38 log10 CFU/g and 3.79 log10 CFU/g, respectively. The yeasts and molds were eliminated from the cheese for 14d at all of the tested OPE concentrations (0.01% to 0.08%). Fortification of cheese with OPE alleviated the increase in titrable acidity in cheese during storage, but the fortified cheese exhibited sensory attributes similar to those of the control cheese. It can thus be concluded that fortification of PWC with OPE improves the chemical and microbial attributes of the cheese.

Keywords: Olive Pomace, Pasteurized white cheese, Total Polyphenol, antioxidant, antimicrobial.

INTRODUCTION

The olive agri-food chain constitutes the largest agro-industrial sector in the Mediterranean basin and covers over five million hectares in the European Union member states (Gómez-Muñoz *et al.,* 2012). Olive oil consumption increased worldwide surpassing three million tons per year during the last ten years. For example, in Jordan, it is about 20,706 Tons/ year (Department of Statistics, Agriculture; Jordan 2017; Mattas and Tsakiridou, 2017). The olive oil extraction process generates a considerable quantity of by-products that range from 2.75 to 4 tons for each ton of oil (Conterno *et al*., 2019). About 37,466 tons of olive pomace is produced in Jordan each year and depend on both fruit quality and extraction technology (Department of Statistics, Agriculture; Jordan 2017). The traditional oil

* Corresponding author.E-mail: g.mehyar@ju.edu.jo

extraction method generates three phases, two of which are by-products (olive cake and wastewater representing 30% and 50%, respectively of the olive fruits) and olive oil about (20% of the olive fruits) (Klen and Vodopivec, 2012). The two-phase extraction system generates olive oil and one by-product which is a combination of liquid and solid (olive cake and wastewater) (Vlyssides *et al.,* 1998). Olive pomace (olive cake or olive husk), is a semi-solid paste, mainly composed of fragments of olive skin, pulp and stone (solid phase), water, and oil (Nunes *et al.,* 2016). It is a phytotoxic and non-biodegradable biomass that is challenging to treat due to its high moisture level (higher energy demand and consequently higher costs during the drying process) and other complex organic compounds (adverse effects on soil) (Salomone and Ioppolo, 2012). Olive pomace is harmful to the environment despite its being a heterogeneous mixture and a significant source of bioactive compounds such as fiber, minerals, polyunsaturated fatty acids, and phenolic compounds (Galanakis, 2011). Phenolic compounds are the main antioxidation compounds in olive pomace (≈98%, namely hydroxytyrosol and its derivatives) (Reboredo-Rodríguez *et al.,* 2017). For instance, ~ 5000 kton/year of olive pomace residue is produced in Spain, with an average of 900 g/ton of total phenolic compounds (Panzella *et al.,* 2020). The anti-oxidation activity is based on preventing the formation of free radicals involved in oxidation processes by donating hydrogen atoms or electrons from elements having this activity (Cassano *et al.,* 2017). In addition, the phenolic compounds have been shown to have antiviral, antimicrobial, antioxidant, anti-inflammatory, and anti-carcinogenic activities (Chanioti *et al.,* 2017). Recovering a polyphenol-rich extract from olive pomace—a low-cost and widely available by-product— constitutes a key point for the valorization of food additives, particularly antioxidants and antimicrobial extracts from natural sources. This recovery would also support a more sustainable bio-economy, reducing environmental problems caused by these wastes and making them suitable for commercialization and development of new food additives (Barba *et al.,* 2016).

Foodborne illnesses related to the consumption of white brined and related cheeses that are contaminated with pathogens and/or their toxins have been recently documented and pose a threat to public health. Moreover, such quality defects in cheese can lead to significant economic losses (Lindqvist *et al.,* 2002). Cheese is among widely consumed foods worldwide (FAO, 2015). A great deal of research has been carried out on the chemical, microbiological, production, and sensory aspects of soft white cheese (Humeid *et al.,* 1986, Haddadin., 2005, Humeid *et al.,* 1990). In this regard, the cheese industry must address the issues of product safety, quality, and shelf-life. Therefore, the main objective of the present study was to investigate the efficiency of using dried olives pomace extract (OPE) as a source of natural antimicrobial and antioxidation components and to investigate the effect of the addition of OPE on the quality and sensory properties of pasteurized white cheese (PWC).

MATERIALS AND METHODS

Pomace collection

Raw olive pomace (about 05 kg) was collected in Balqa-Salt in Jordan from the Aljazzazi Olive Oil Mill in December 2021. Olive pomace was homogenized and collected in polyethylene bags (2.5kg for each bag) and stored in a freezer at -20°C. Before use, the pomace samples were thawed for 24 hr under refrigeration temperature (5°C).

Pomace drying process

One to two kilograms of pomace were dried in an oven at 40°C for 6hr with constant stirring every 15 min until reaching a fixed weight. The dehydrated samples were then packed in polyethylene bags, placed in closed plastic containers, and kept refrigerated at 8°C in a dark place for further analysis.

Biochemical composition of olive pomace

Proximate analyses of dried olive pomace were performed in triplicates and expressed as g/100 g of dry matter (DM). Moisture content was determined using a

direct drying method in a hot air oven (Memmert, Karlklob-West Germany) at 105°C for ~4hr until a constant weight was attained (AOAC,1990). The Ash content level of dried olive pomace was determined by the dry ashing method using a muffle furnace (Carbolite ™ CWF 1100; Germany) at 550°C for 6 hr (AOAC, 2011). Soxhlet extractions were performed to determine crude fat content using diethyl ether as a solvent with the Soxhlet extraction apparatus (Electrothermal EME60250/CEBX1; UK) (AOAC, 2000). Protein contents of pomace were determined using the Kjeldahl method with a conversion factor of 6.25 (Protein % = Nitrogen % x 6.25) by a heating digester (VELP Scientifica DK 20, Italy) and behr distillation (behr Labor-Technik, Germany) (AOAC, 2011). The crude fiber content of dried pomace was obtained using an ANKOM$^{200/220}$ fiber analyser (ANKOM, USA) and filter bags (ANKOM bags F57) according to the Van Soest *et al.,* (1990) method. Total soluble carbohydrates were determined by calculating the percent remaining after all the other components had been measured (Tang, 2003):

% Soluble carbohydrates =
100 - ٪moisture - ٪protein - ٪lipid - ٪mineral - ٪fiber.

Preparation of olive pomace extracts (OPE)

The dried olive pomace was extracted using two different extracting solvents including ethanol and water (75:25, soln. A), methanol and water (75:25, soln. B) (Al-Ismail *et al.,* 2006). The weighed dried olive pomace sample (10g) was mixed with 100 ml of each (solutions-A or B) and kept under agitation in a water bath for 1hr at 40°C, sonication in an ultrasonic bath (ISO LAB, Germany) for 30min at 40°C, then filtered with Whatman filter paper No. 4. The filtrated solution was evaporated by a rotary evaporator at temperatures corresponding to each solvent (78 °C for ethanol and 64 °C methanol). The residues (as paste) were then transferred in petri dishes to the oven at 40°C to evaporate any solvent. These residues were weighed (11 g) and then dissolved in distilled water in a volumetric flask and the total volume was made up to 200 ml in order to obtain a 5.5% (g/ml) stock solution. For enhanced purity, the resultant mixture was centrifuged at 4000 RPM for 15 min (Hettich, D-78532, Germany). The supernatant was then collected and stored at refrigeration temperature (~5°C) in dark closed bottles during which it was examined for its antioxidant and antimicrobial activities for further analysis. The average yield content (g/100g DW) of olive pomace extracts (OPE) was determined in triplicate.

Determination of Antioxidants

Total Phenolic content

The total phenolic compounds were determined according to Al-Ismail *et al.,* (2006). The phenolic compounds present in OPE (ethanol or methanol) were determined by the addition of 0.5ml of the Folin-Ciocalteu Reagent (FCR). The sample (2.5 µl) was left to stand for 60 min and then measured at 760 nm using a spectrophotometer (Biotech Engineering Management Co., UK). Gallic acid was used as the standard for a calibration curve. The total phenolic compound contents (mg/100g) were expressed as Gallic acid equivalent (GAE).

Antioxidant activity "DPPH Free Radical Scavenging Ability Assays"

DPPH radical scavenging effect was determined using DPPH (2, 2-Diphenyl-1-picrylhydrazyl) with different concentrations of each OPE, the absorbance of OPE was measured at 517 nm using a spectrophotometer (Biotech Engineering Management Co., UK) after placing the samples in the dark for 30min (Al-Ismail *et al.,* 2006). IC_{50} (the concentration of extract in mg/ml needed to scavenge 50 % of the DPPH radical) was calculated from the concentration-response curves.

Determination of functional compounds of olive pomace by the HPLC-MS analysis

Pomace extraction process

The dried olive pomace sample was extracted by methanol with a constant solid–liquid ratio of 1:10 according to Aliakbarian *et al.* (2011). Testing of the extracts was performed in a high-pressure–high-

temperature reactor (model 4560, PARR Instrument Company, Moline, IL, USA), which contained appropriate valves to allow the introduction and removal of gasses inside the reaction chamber to avoid phenolic oxidation. All tests were carried out under a nitrogen atmosphere by flushing nitrogen through the reactor for 2 minutes. The alcoholic extracts were then centrifuged in a PK131 centrifuge (ALC, Alberta, Canada) at 6000g for 10 min. and supernatants were subjected to quantitative analyses.

Identification of phenolic compounds in OPE

Free phenolic compound identification was performed according to Aliakbarian *et al.,* (2011) by high-performance liquid chromatography (model Shimadzu ExionLC), using a UV detector (at 280 nm) and a C18 reverse-phase column (model 201TP54, Vydac, Hesperia, CA, USA) with a C18 guard column (Alltech Associates, Inc., Deerfield, IL, USA). Samples were filtered through 0.20 µm membranes (Sartorius Stedim Biotech GmbH, Goettingen, Germany). Separation was achieved using a linear gradient of two solvents: solvent A (0.1% formic acid in water) and solvent B (acetonitrile). The injection volume was 10µl, and the evaluation of each phenolic compound expressed as mg/100g olive pomace, was based on the peak in comparison with standard solutions. Mass spectroscopy was performed in a Turbo Ion Spray model X500 QTOF source in a positive mode according to de la Torre-Carbot *et al.* (2005) with the following settings: capillary voltage 5000 V, nebulizer gas (N2) 50, curtain gas (N2) 30, collision gas (N2) 7. Drying gas was heated to 550 °C and introduced at a flow rate of 5000 cm^3 min^{-1}.

Processing of pasteurized white cheese

Pasteurized white cheese (PWC) was produced in the dairy processing pilot plant of the Department of Nutrition and Food Technology, University of Jordan, using the traditional method for pasteurized cheese production (Humeid and Tukan., 1986).

Fortification and treatment of PWC with OPE

OPE was added to the prepared PWC to achieve levels (0.01٪, 0.02٪, 0.03٪, 0.04٪, 0.05٪, 0.06٪, 0.07٪ and 0.08; w/w٪) after the casein was coagulated and drained for 5-8 min. but before the final pressing. The mixtures were mixed with a sterilized spatula under a laminar flow cabinet. A similar sample was prepared but without the OPE addition and stored under the same storage conditions (control). Samples were stored at refrigeration temperature (5°C) for 1 month, and sampled at 24hr, 7d, 14d, 21d, and 30d for physiochemical and microbiological analysis.

Total Phenolic Compound content in PWC treated with OPE

Ten grams of each cheese sample were mixed with 10 ml of methanol solution (75 methanol and 25 ml distilled water) and extracted for 30 min at 50°C in a water bath (Raypa Thermostatic bath; Barcelona, Spain). The mixture was cooled and filtered by cheesecloth, and the residues were washed with 2ml of the same solvent solution. The obtained extracts were immediately analyzed (within 10 min). Total phenolic compounds were determined in duplicate by UV-vis spectrophotometry according to the Folin-Ciocalteu method (Spinelli *et al.,* 2015). The total phenolic content was expressed as mg of gallic acid equivalents (GAEs) per g of dry weight (DW).

Microbiological analysis

Initial suspension and decimal reduction

Decimal dilutions (from 10^{-1} to 10^{-4}) from the control and treated PWC samples were prepared using sterilized peptone water (prepared to contain 1.0% peptone and 0.5% NaCl, Oxoid CM0009) at ambient temperature according to ISO 6887-1:1999 method.

Microbial analysis

Mesophilic aerobes, total lactic acid bacteria, yeasts and molds, and *Staphylococcus aureus* counts were determined according to the ISO methods: EN ISO 4833:1-2013, ISO 15214:1998, ISO 21527-1:2008 and

ISO method 6888-1; 1999, respectively. The media used were plate count agar, MRS agar, potato dextrose agar (supplemented with chloramphenicol and chlortetracycline-HCl), and Baired-Parker agar medium supplemented with potassium telluite and egg yolk. All media and supplements were from Oxoid Limited (UK, Hampshire).

Confirmation Tests of *S. aureus*

Coagulase test:

Representative colonies of *S. aureus* were inoculated into BHI broth (Himedia, India) and incubated for 18-24 hr at 35 ±1°C. An amount of 0.5 ml reconstituted coagulase plasma with EDTA (Liofilchem, Italy) was added to BHI culture (1ml) and mixed thoroughly, incubated at 35 ±1°C, and then examined periodically over 6-h for clot formation. Only firm and complete clots that remained in place when the tube was tilted or inverted were considered positive for *S. aureus.*

DN_{ase} test

DN_{ase} agar plates were prepared, dried, and inoculated with representative colonies of *S. aureus* with a sterile inoculating loop. The plates were then incubated at 35-37°C for 24 hr, flooded with a 1N HCl solution, and the excess acid was topped off. Plates containing a clear zone around the colonies within 5 min were considered positive.

Chemical analysis of PWC

Titrable acidity

The Titrable acidity of cheese was measured according to the AOAC method (920.124 (2011). TA expressed was as % of lactic acid.

Lipid oxidation: thiobarbituric acid (TBA) procedure

Ten grams of a homogeneous sample were weighed and combined with 25 ml of 20٪ trichloroacetic acid (TCA) and 20 ml of warm water (Raharjo *et al.,* 1993). The mixture was then homogenized in stomacher bags (Model AES, Labprat) for 2 min, filtered through Whatman filter paper no 1 after which 2 ml of filtrate was combined with 2 ml of 0.02M aqueous 2 thiobarbituric acid (TBA) in a test tube. The tubes were then incubated at 22 -° C in a dark room for 20 hr. The absorbance of the resultant solution was measured spectrophotometrically at 532 nm. The TBA number, expressed as mg of malondialdehyde /kg of the sample (ppm), was calculated by measuring the absorbance of a pink-colored mixture of the TBA-reactive substances at 532nm multiplied by a factor of 7.8.

Sensory evaluation

Sensory analysis of the control and fortified PWC was carried out by 30-panel members (employees and students) after 7d of refrigerated storage (≤ 5°C) (ISO 4121, 2003). Food taste panelists were asked to use the 9-point hedonic scale for overall acceptability, softness, color, taste, and flavor. PWC samples and the control were desalted by soaking in potable tap water overnight in order to reduce the salt content.

Statistical analysis

Statistical calculations were performed -Analysis of variance ANOVA- using the SAS program, 2011 (SAS Institute Inc., Cary, NC). Significant differences among means of treatment were determined using the LSD tests. Differences at $P \leq 0.05$ were considered to be significant. All treatments were conducted in duplicate or triplicate.

RESULTS AND DISCUSSION

Proximate analysis of olive pomace

Fiber represented the major constituent (60.85 g/100 g DM) followed by crude fat (11.91g/100 g DM), crude protein, and moisture (6.83 and 8.46 g/100 g DM, respectively) (Table 1). The ash and carbohydrate contents were 5.18 g/100 g DM and 6.77 g/100 g DM, respectively. These values are similar to those reported by other authors (Haddadin *et al*., 2009; Uribe *et al*., 2014; Zhao *et al.,* 2022). It was reported that climatic factors can influence the chemical composition of olive pomace as well as other factors such as the agricultural practices, cultivar, the ripening stage, and the process of oil

extraction (Portarena *et al.,* 2017). Olive pomace contains the remaining olive fat, water, and a large number of bioactive compounds, which can have further valuable and novel applications (Roselló-Soto *et al.,* 2015).

Table 1: Proximate analysis of dried olive pomace (g/100g DM[1])

Component	Mean± SD[2]
Moisture	8.46±0.11
Ash	5.18±0.31
Fiber	60.85±0.42
Fat	11.91±0.67
Protein	6.83±0.04
CHO[3]	6.77±0.23

[1]DM: Dried Matter

[2]Each value is the mean of three replicates ±SD (Standard Deviation)

[3]CHO: Carbohydrates

Yield of olive pomace extracts (OPEs)

Methanol extraction had significantly (P≤0.05) higher yield (23.40 g/100g DW) than ethanol extraction (22.18 g/100g DW) (Table 2). These results are similar to those obtained by other authors (Badawy and Smetanska, 2020; Gómez-Cruz *et al.,* 2020; Zhao *et al*., 2022). The selection of solvents could be a crucial factor in the efficiency of extraction of the bioactive compounds from biomass (De Jesus *et al*., 2020). Solvents such as ethanol, acetone, and ethyl acetate have been widely used owing to their low toxicity and their being allowed by the European Food Safety Authority (EFSA) for fortification of foods with the extracted functional ingredients (Gullón *et al.,* 2018). It was reported that the use of water with an organic solvent contributes to the creation of a moderately polar medium that ensures the extraction of phenolic compounds, providing better results compared to using a pure organic solvent (Musa *et al.,* 2011). Methanol has high polarity which could produce high extraction yields. However, its toxic characteristic could be a limiting factor when it is used in food and pharmaceutical applications (Hassim *et al*., 2014). Although the extraction yield of ethanol is significantly lower than ethanol, it has less toxicity and is considered a green, safe, and nontoxic media for extraction compared to other organic solvents such as methanol (Rodríguez-Rojo *et al.,* 2012). Thus, ethanol was chosen to extract the olive pomace to be used in PWC.

Determination of Antioxidants

Total Phenolic content

The methanol extraction contained higher total phenolic content than the ethanol extraction with amounts of 5958.0 mg GAEs/100g DW and 5587.4 mg GAEs/100g DW, respectively (Table 2). These results are comparable to those obtained by Alhamad *et al.,* (2017), Nunes *et al.,* (2021), and Uribe *et al.,* (2014) by using several solvents to extract phenolic compounds from olive or olive pomace. Olive pomace is considered to be an important source of phenolic compounds since only 1–2% of the phenolic compounds in olives go into olive oil through its mechanical extraction process (centrifugation of oil paste), while 53% and 45% of these compounds remain in the liquid waste and the solid by-product (olive pomace), respectively (Chanioti *et al*., 2017). It was reported that the extraction efficiency of the phenolic compounds from olives and the antioxidant potential is affected by the operation parameters such as the extraction temperature, the extraction time, the liquid-to-solid ratio (L:S), and the type of solvent (Sahin *et al.,* 2013). Polyphenolic yields were found to increase with increasing the polarity of the solvents (from polar to non-polar) as a result of stronger interactions (hydrogen bonds) between the polar sites of the phytochemical compounds and the solvent. The more the hydroxyl groups in the molecule, the higher the polarity (Liu *et al*., 2007).

Diphenyl-1-Picrylhydrazyl Radical (DPPH•) scavenging ability

DPPH assay was calculated as IC_{50}, the number of extracts that will react with 50% of the added DPPH radicals in the total volume of the assay under the given reaction conditions (Piaxao *et al.* 2007). From Table 2, the IC_{50} of OPEs by methanol/water (75/25) was significantly (P ≤ 0.05) higher (1.61 µg/ µg DPPH) than

those obtained by ethanol/water (75/25) (1.56 µg/ µg DPPH) (Table 2) so ethanol extracts yielded the highest DPPH radical scavenging activity. The strong scavenging activity of the former could be related to the high concentration of antioxidant compounds in ethanol extract such as hydroxytyrosol, oleuropein, and oleuropein aglycon (Leouifoudi *et al*., 2014). Furthermore, the ethanolic extract possessing a phenolic compound that contains a higher number of hydroxyl groups has a higher antioxidant activity (Leouifoudi *et al.,* 2014). Our IC_{50} could vary from those stated by other authors (Chanioti *et al.,* 2017 and Badawy and Smetanska, 2020) which could be related to the difference in extraction conditions. The scavenging of the 2, 2-Diphenyl-1-Picrylhydrazyl Radical (DPPH) was used to evaluate the antioxidant activity of the OPEs. This method is based on the capability of an antioxidation compound to donate hydrogen radicals or an electron to DPPH radicals in order to produce a stable free radical with a deep violet color. When the odd electron becomes paired to DPPH radicals, the DPPH is reduced to corresponding hydrazine (DPPH-H) and becomes decolorized from its initial deep violet to a light-yellow color. The degree of fall in the absorbance is measured spectrophotometrically and is proportional to the concentration of the antioxidant (Piaxao *et al.* 2007). It was reported that the antioxidant capacity of extracts is affected by the solvent system used (Bandoniene et al., 2022).

Table 2: Average extraction yield, total phenolic content (TPC), and IC_{50}[1] values of DPPH inhibition of olive pomace extract by the two solvents

Solvent	Extraction yield (g/100g)[3]	TPC (mg GAEs[2]/100g DW)[3]	IC_{50} (µg/ µg)[3]
Ethanol	22.18^{b} ±0.05	5587.4^{b} ±174.1	1.56^{b} ± 0.020
Methanol	23.40^{a} ±0.02	5958.0^{a} ±174.1	1.61^{a} ± 0.004

[1]IC_{50}: The half-maximal inhibitory concentration

[2] GAE: Gallic Acid Equivalents

[3]Each value is the mean of three replicates ±SD (Standard Deviation)

Mean within the same column with different subscripts are significantly different at the 5% level of probability ($p \leq 0.05$).

Determination of functional compounds in OPE

A detailed analysis of the OPEs by HPLC/MS and the respective chromatograms obtained at 280 nm are presented in Table 3. The UV chromatography demonstrated eight major signals that were tentatively identified by the retention time and according to mass/charge numbers by ion -m/z ion. The chromatographs were counted per second by comparing their relative retention times and spectral data (UV-vis and MS) with those of corresponding analytical standards. It can be concluded from the results summarized in Table 3 that all the compounds isolated were phenolic compounds and those with the highest concentrations were Syringic acid and p-coumaric (peak 4 and 6, respectively) with counts per second (CPS) of 4000 and 2400, respectively. The third and fourth compounds were vanillin and caffeic acid (peaks 5 and 3 respectively) with counts per second of 1900 and 1200, respectively. Then apigenin and tyrosol (peaks 8 and 1) had CPS of 750 and 700, respectively. Vanillic acid and oleuropein (peaks 2 and 7) with CPS of 600 and 240, respectively were the least contained compounds that were found to belong to the secondary metabolic pool of OPE.

Table 3: Identified compounds by the HPLC/MS^n in the olive pomace extract

Analyte Peak Number	Analyte Peak Name	Count Per Second	Retention time (Min.)	Molecular weight (Da)	Chemical Formula
04	Syringic	4000	0.57	199.0601	$C_9H_{10}O_5$
06	p-Coumaric	2400	3.17	165.05462	$C_9H_8O_3$
05	Vanillin	1900	13.65	153.05462	$C_8C_8O_3$
03	Caffeic acid	1200	0.93	181.04954	$C_9H_8O_4$
08	Apigenin	750	18.23	271.0601	$C_{15}H_{10}O_5$
01	Tyrosol	700	10.61	139.07536	$C_8H_{10}O_2$
02	Vanillic acid	600	8.40	169.04954	$C_8H_8O_4$
07	Oleuropein	240	15.90	558.21812	$C_{25}H_{32}O_{13}$

Numerous papers in the literature have reported the detailed composition of OPEs from various cultivars/regions/countries. It was described that the total polyphenol contents and its derivatives are considered to represent a type of trademark of this matrix (Difonzo *et al.,* 2021C). All compounds identified in the olive pomace belong to the class of polyphenols, as widely reported in the literature. Extraction solvent also plays a pivotal role in the selection of extracted compounds, together with extraction time and temperature (Irakli *et al.,* 2018). Moreover, the European Food Safety Authority (EFSA) has issued a favorable scientific opinion regarding health claims for the dietary consumption of polyphenols and their derivatives (protection of blood lipids from oxidative damage) (EFSA, 2011). Other studies have reported that polyphenol derivative has antioxidant, anti-microbial, anti-inflammatory, and anti-diabetic activity (Salah *et al.,* 2012). These results would indicate that all compounds defined in the current study were found in previous researche (Aliakbarian *et al.,* 2011; Zhao *et al.,* 2022; Ribeiro *et al.,* 2020).

Analysis of fortified pasteurized white cheese (PWC) with OPE

Some researchers have demonstrated the positive effect of OP addition on the properties of different food products (Conterno *et al.,* 2019). However, few studies have reported its incorporation in dairy products such as milk and yogurt (Cho et al., 2020). Our study was thus the first on the fortification of pasteurized white cheese (PWC) with OPEs.

Total Phenolic Compound content in PWC treated with OPE

Fig. 1 shows that the addition of OPE (0.02 % to 0.08%) to PWC significantly ($P \leq 0.05$) increased the total phenolic content in PWC compared to the control. High amounts of phenolic compounds were found in PWC with high concentrations (0.08%, 0.07%, and 0.06%) of OPE with the amount of total phenol equal to 2836.98, 2538.90 and 2433.13 mg GAEs/100g respectively. It also demonstrates a significant similarity between samples with low concentrations (0.01%, 0.02%, and 0.03%) with amounts of 1077.37, 1086.98, and 1269.67 mg GAEs/100g, respectively, and the control sample (904.29 mg GAEs/100g). These results are similar to those obtained by other authors (Simonato, *et al.,* 2019); (Ribeiro *et al.,* 2021); (Balli *et al.,* 2021); (Cecchi *et al.,* 2019).

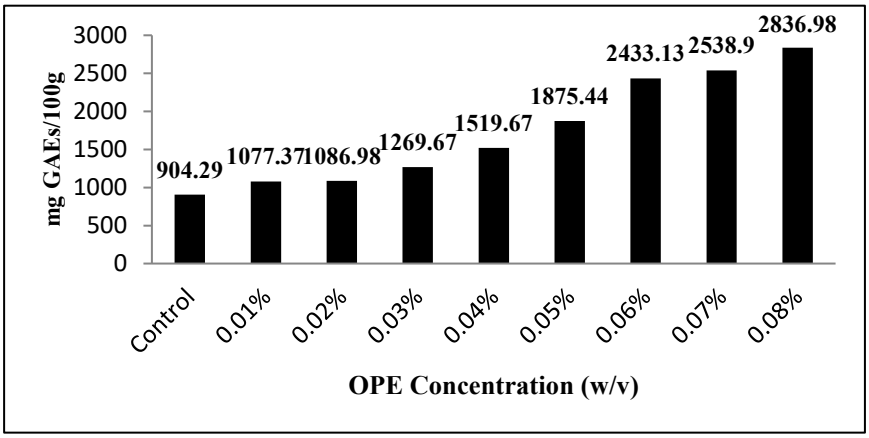

Figure 1: Effect of fortification of pasteurized white cheese with different concentrations of olive pomace extract on the content of total phenolic compounds

The presence of relatively low concentrations of total phenolic compounds in the control (PWC without OPE) could possibly be due to the presence of low molecular weight antioxidant compounds in milk components such as polyphenols that are affected by animal feeding and heat treatment of the milk (Chouchouli *et al.,* 2013). The low levels of total phenolic compound values in the PWC fortified with low concentrations of OPEs (from 0.01% to 0.03%) may diminish during the storage period. This is due to interactions of these phenolic compounds with the small amounts of monounsaturated fatty acid (25%) and polyunsaturated fatty acid (2.5%) in cheese which can act to protect lipid peroxidation by retarding reaction with hydrophilic radicals (Lindmark, 2008). This could lead to the eventual loss of the ability of phenolic compounds to prevent oxidation of the unsaturated fatty acid (UFAs') in the PWC, which makes the product more vulnerable to rancidity. This could explain the lower amount of TPC in PWC treated with low concentrations of OPE (Gorelik *et al.,* 2013; Jakobek, 2019). Abdel-Aal and Rabalski (2013) suggested that the decrease in bioactive compounds in a food product with storage depends on several factors including -the type of product, the recipe, processing conditions, and the phenolic compound content of the food product.

Microbiological analysis

Mesophilic aerobes

It should be noted that immediately after the cheese production (up to 1d), the control and the fortified PWC with a low concentration of OPE (from 0.01% to 0.05%) had higher total mesophilic aerobes with log mean counts of 3.44 $\log_{10}$CFU/g and 3.23 $\log_{10}$CFU/g, respectively. The mesophilic aerobes count was significantly ($P<0.05$) lower (2.97 $\log_{10}$CFU/g to 2.72 $\log_{10}$CFU/g) as a result of adding a high concentration of OPE (0.06% to 0.08%), respectively. After 7d of storage, there was no significant increase in the mesophilic counts in both the control and treated samples. However, the counts began to increase again at higher rates especially at lower concentrations of OPE (0.01% to 0.05%) and the control until a month of refrigeration storage (4.04 $\log_{10}$CFU/g). Samples with high concentrations of OPE (0.06% to 0.08%) maintained low levels of mesophilic aerobes counts (3.74 $\log_{10}$CFU/g to 3.61 $\log_{10}$CFU/g) after 30d of refrigerated storage (Fig. 2). The mesophilic count development in the control samples was a result of the normal manifested microbial growth in dairy product due to poor hygiene conditions and a lack of refrigeration (Haddad *et al.,* 2017). It was reported that the inhibitory activity of the OPE in the fortified PWC with a high concentration of OPE was greater against Gram-positive bacteria (*L. innocua* and *S. aureus*) than Gram-negative bacteria (Gómez-Cruz *et al.,*

2020, and Nunes *et al.,* 2021). This would be due to the fact that the polyphenol structure (hydroxyl group –OH interacts with the microbial cell membrane resulting in cell death –membrane disruption-) (Xue and Zhong, 2013). However, the Gram-negative bacteria (*E. coli* and *Salmonella* sp) are rich in lipopolysaccharides that restrict the penetration of foreign molecules and increase the resistance of the bacteria to these antimicrobial agents (Harkat-Madouri *et al.,* 2015).

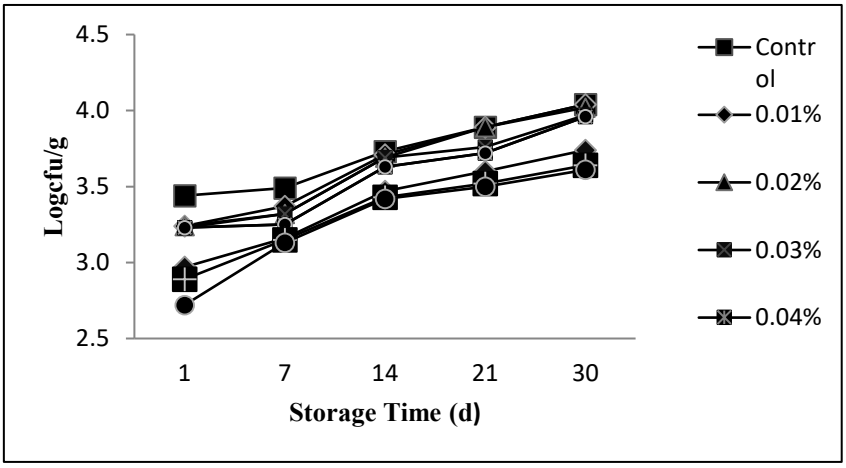

Figure 2: Effect of fortification of pasteurized white cheese with different concentrations of olive pomace extract on its mesophilic aerobic count (log_{10}CFU/g) during refrigerated storage (5°C)

Lactic Acid Bacteria (LAB)

Immediately after PWC production (up to 1d), the control PWC and the fortified PWC with a low concentration of OPE (from 0.01% to 0.05%) had higher total LAB with mean counts of 3.02 log_{10} CFU/g and 2.86 log_{10} CFU/g, respectively. However, the counts decreased significantly ($P<0.05$) to 2.48 log_{10} CFU/g by adding a high concentration of OPE (0.06% to 0.08%). After 14d of storage, there was a significant increase in the LAB in both the control and treated samples with low concentrations of OPE (0.01% to 0.06%) up until the end of the refrigerated storage, where the control had the highest count (3.79 log_{10}CFU/g). Samples treated with higher concentrations of OPE (0.06% to 0.08%) elicited lower levels of LAB counts (3.38 log_{10}CFU/g) during the 30 d storage period (Figure 3). OPEs inhibited the growth of LAB (antimicrobial effect against Gram-positive bacteria) (Gómez-Cruz *et al.,* 2020, Nunes *et al.,* 2021). The antimicrobial effectiveness of oleuropein and other phenolic compounds found in OPE has been proven mostly against various bacteria including the lactic acid bacteria, namely: *Lactobacillus plantarum*, *L. brevis,* and *Leuconostoc mesenteroides,* and against bacteria associated with infections of the human intestinal or respiratory tracts (Soler-Rivas *et al.,* 2000; Fleming *et al.,* 1973).

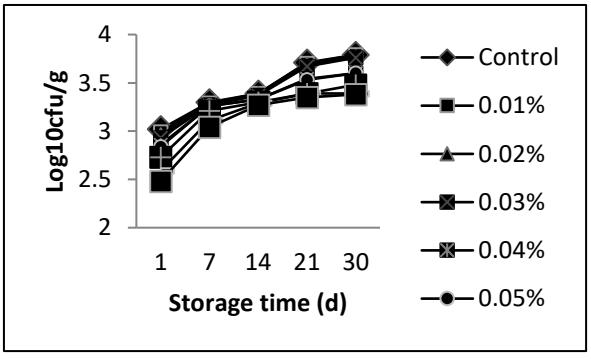

Figure 3: Effect of fortification of pasteurized white cheese with different concentrations of olive pomace extract on its lactic acid bacteria count (log10CFU/g) during refrigerated storage (5°C)

Yeasts and Molds counts

Untreated PWC (control) and the fortified PWC with all concentrations of OPE (from 0.01% to 0.08%) had undetectable counts of yeasts and molds for up to 14d of storage (Figure 4). After 21 and 30d of refrigerated storage, there was a significant increase in the yeasts and molds counts in both the control and treated samples with low concentrations of OPE (0.01% to 0.05%, respectively). The counts were kept below 2.4 $\log_{10}$CFU/ g and 2.7 $\log_{10}$CFU/ g in treated samples and in the control after 30d of storage, respectively. Winkelhausen *et al.* (2005) demonstrated that the phenolic compounds derived from olive pomace have good potential as a natural fungicide against common pathogens, especially against *Alternaria solani, Botrytis cinerea,* and *Fusarium culmorum*.. The authors attributed the antifungal activity of the OPE to the aldehyde moiety in the vanillin. Additionally, vanillin was reported to pose a remarkable antimicrobial activity against (*Saccharomyces cerevisiae*, *Zygosaccharomyces bailii,* and *Zygosaccharomyces rouxii* (Fitzgerald, 2003).

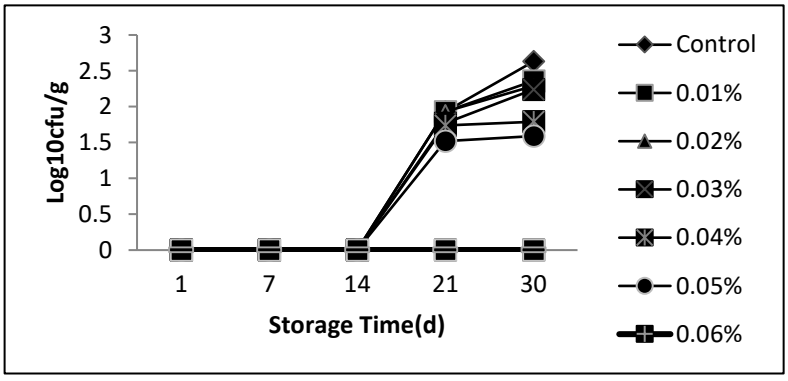

Figure 4: Effect of fortification of pasteurized white cheese with different concentrations of olive pomace extract on its yeast and molds counts (log10CFU/g) during refrigerated storage (5°C)

***Staphylococcus aureus* count**

All of the treated samples and control PWC did not contain detected *S. aureus* (data is not shown) as all the grown colonies gave negative coagulase and DNase testes. However, it was demonstrated that OPE components have an inhibitory effect OPE especially against Gram-positive bacteria (*L. innocua* and *S. aureus*) (Gómez-Cruz *et al*., 2020 Nunes *et al.,* 2021). Yakhlef *et al*. (2018) demonstrated the bactericidal effect of olive mill wastewater, which contained high concentration of phenolic (10.828 mg/L) with minimal bactericidal concentration (MBC) for all target microorganisms (*Pseudomonas fluorescens* (CECT 378), *Staphylococcus aureus* (CECT 239), *Escherichia coli* (CECT 434), and *Enterococcus faecalis* (CECT 481) was 2.5%. The absence of *S. aureus* in the PWC could be due to cheese pasteurization that eliminates all known milk-borne pathogens (Alegbeleye *et al.,* 2018).

Chemical analysis of cheese

Titrable acidity

In general, there was a significant (P≤0.05) decrease in titrable acidity of fortified PWC with increasing OPE concentration throughout the 30d of refrigerated storage. The control samples maintained significantly (p ≤0.05) higher titrable acidity compared to all treated samples at each particular sampling time (Figure 5). In addition, there was a significant (P≤0.05) increase in titrable acidity within each PWC treatment and the control during the 30d storage period to a maximum of 0.183% (Figure 5) due to lactose fermentation and the formation of lactic acid and other organic acids in the cheese. Aini *et al.* (2020) and

Elewa *et al.* (2009) observed the development of acidity in different kinds of cheese during the refrigeration period as a direct response to converting the residual lactose into lactic acid by the available micro-flora. These results are concomitant with the results of lactic acid bacteria count (Figure 3), where LAB counts decreased with an increase in OPE concentration, thereby producing less lactic acid (Kim *et al.,* 2017).

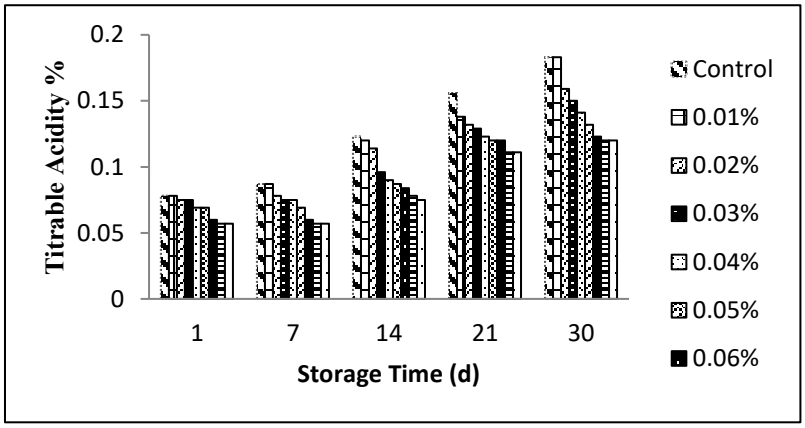

Figure 5: Effect of fortification of pasteurized white cheese with different concentrations of olive pomace extract on its titrable acidity during refrigerated storage (5°C)

Lipid oxidation of cheese

In general, there was a significant (P≤0.05) decrease in the lipid oxidation (as the TBA value) of fortified PWC with increasing OPE concentration throughout the refrigerated storage of 30d. The control samples maintained significantly (p ≤0.05) higher lipid oxidation levels compared to the treated samples at each particular sampling time. In addition, there was a significant (P≤0.05) increase in lipid oxidation within each PWC treatment during the 30d storage period to a maximum of 0.746% (Figure 6). Table 5 shows that the OPE consisted of a number of aromatic compounds such as hydroxytyrosol, tyrosol, caffeic acid, oleuropein, and oleuropein aglycon which may have antioxidation activity (Leouifoudi *et al.,* 2014). Phenolic compounds are secondary plant metabolites that have the ability to act as antioxidants by neutralizing reactive oxygen and nitrogen species by their hydroxyl groups, thereby making these compounds good hydrogen donors. Moreover, phenolic compounds can moderate free radical generation by chelating metal ions (Gómez-Cruz *et al.,* 2020). Gülçin, (2005) found that caffeic acid at concentrations of 10 and 30g/mL showed 68.2 and 75.8% inhibition of lipid peroxidation in linoleic acid emulsion, respectively. The significant increase of TBAs in the control sample and samples with low concentrations of OPE was the result of oxidation of the product by several factors such as light on fat and the level of oxygen in the heavily brined solution (Al-Ismail *et al.,* 2003, Mehyar *et al*., 2012). Moreover, the low surface/volume ratio of the cheese pieces and the low diffusion rate of the dissolved oxygen through these pieces could have led to minimum contact of the fat with the dissolved oxygen, thus preventing excessive oxidation (Al-Ismail *et al.,* 2003). Our results were similar to those obtained by other authors (Vitali Cepo *et al.,* 2018) and (Innosa *et al*., 2020).

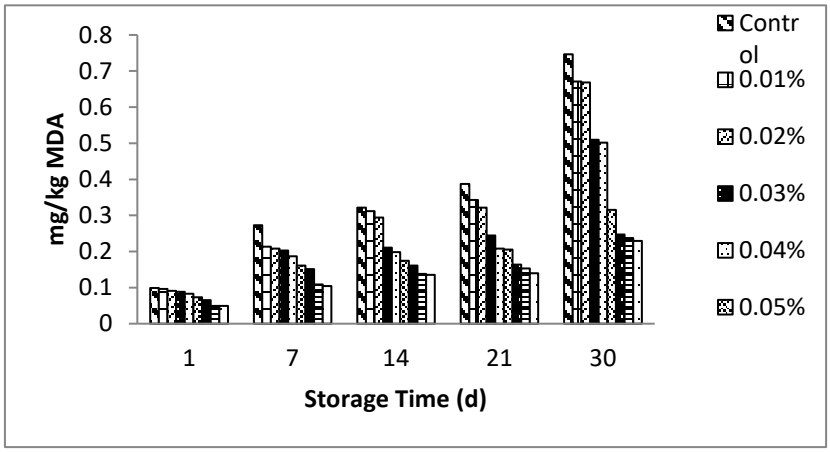

Figure 6: Effect of fortification of pasteurized white cheese with different concentrations of olive pomace extract on lipid oxidation (expressed as mg/kg of malondialdehyde, MDA) in PWC as determined by thiobarbituric acid assay

Sensory evaluation

In general, there were no significant (P>0.05) differences in sensory properties between the OPE-treated samples and the control. However, the PWC color in the OPE-treated samples was significantly better compared to the control. In addition, although there were no significant (P>0.05) differences within each PWC treatment during the 30d storage period, significant improvement in softness was observed at a higher concentration of OPE (0.08%). The significant difference in color of the PWC-treated samples at concentrations of 0.01%, 0.04%, and 0.05% compared to the control could be attributed to the oxidation of fats. In the PWC control, this resulted in a transition from white to a more yellowish color. The significant increase in softness on most fortified pasteurized PWC compared to the control sample could be due to the decomposition of the cheese protein as a result of the increase in bacterial activity at the beginning of the second week, especially in the absence of OPE (the control). Despite the slight changes in color and softness, the fortified PWC attained approximately the same overall acceptability scores as the untreated PWC sample. It can thus be concluded that the addition of OPE did not result in significant sensory changes to the product from the standpoint of consumer acceptance (Figure 7). These results are compatible with those provided by other authors (Lin *et al.,* 2017); (Badawy and Smetanska, 2020); (Cecchi *et al*., 2019) and (Simsek *et al.,* 2021).

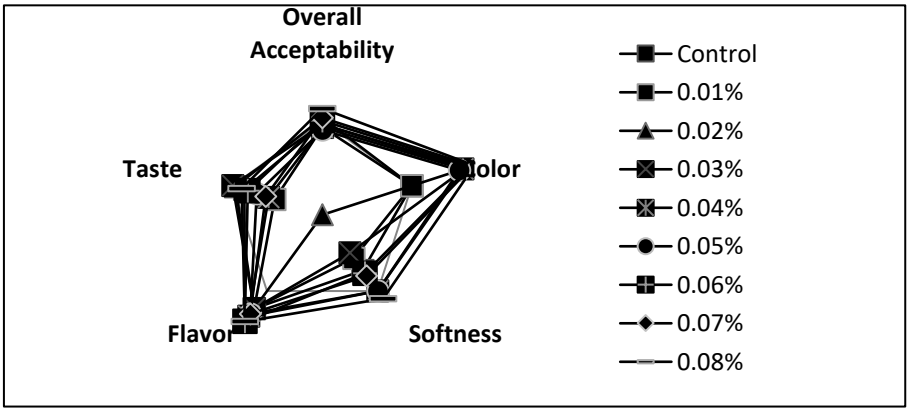

Figure 7: Sensory evaluation of PWC with OPE at day 7 during refrigerated storage (5°C)

Conclusions

Olive pomace is rich in phenolic compounds that have high antioxidation and antimicrobial activities. Incorporation of olive pomace extract in pasteurized white cheese enriched the cheese with phenolic compounds and slightly improved the microbial quality of PWC. Treatments of PWC with OPE retarded lipid oxidation and improved the sensory properties of the cheese. This study thus demonstrated the effectiveness of incorporating olive pomace in the PWC. Additional research is warranted in order to explore the antimicrobial activity of OPE and their minimum inhibitory concentration in PWC against various common foodborne pathogens.

Acknowledgment

The authors would like to thank the Deanship of Graduate Studies at Jordan University for their financial support of this research and the Aljazzazi Olive Oil Mill in Balqa-Salt in Jordan for providing the olive pomace.

REFERENCES

Abdel-Aal, E. S. M., & Rabalski, I. (2013). Effect of Baking on Free and Bound Phenolic Acids in Wholegrain Bakery Products. *Journal of Cereal science*, 57(3):312-318.

Aini, N., Sustriawan, B., Prihananto, V., Sumarmono, J., Ramadan, R. N., & Romadhon, D. (2020). Formulation of Low-Fat Cheese Analogue from Sweet Corn Extract using Papain and Lime Extract as Coagulant. *Food Research*, 4(4): 1071-1081.

Alegbeleye,O. O., Guimarães, J. T., Cruz, A. G., & Sant'Ana, A. S. (2018).Hazards of a 'Healthy'Trend? An Appraisal of the Risks of Raw Milk Consumption and the Potential of Novel Treatment Technologies to Serve as Alternatives to Pasteurization. *Trends in Food Science & Technology*, 82: 148-166.

Alhamad,M. N., Rababah, T. M., Ereifej, K., Esoh, R., Feng, H., & Yang, W. (2017).The Physicochemical Properties, Total Phenolic, Antioxidant Activities, and Phenolic Profile of Fermented Olive Cake. *Arabian Journal of Chemistry,* 10(1): 136-140.

Aliakbarian,B., Casazza, A. A., & Perego, P. (2011).Valorization of Olive Oil Solid Waste using High Pressure–High Temperature Reactor. *Food Chemistry*, 128(3): 704-710.

Al-Ismail,K. M., & Humied, M. A. (2003). Effect of Processing and Storage of Brined White (Nabulsi) Cheese on Fat and Cholesterol Oxidation. *Journal of the Science of Food and Agriculture,* 83(1), 39-43.

Al-Ismail,K., Hamdan, M., & Al-Delaimy, K. (2006).Antioxidant and Anti-Bacillus Cereus Activities of Selected Plant Extracts. *Jordan Journal of Agricultural Sciences*, 2(2): 64-74.

Association of Official Analytical Chemists, International (1990), Official Methods of Analysis, 15th ed. Kenneth Helrich, USA: AOAC INTERNATIONAL.

Association of Official Analytical Chemists, International (2000), Official methods of analysis Vol, 17th ed. Arlington, VA: AOAC, INTERNATIONAL.

Association of Official Analytical Chemists, International (2011), Official Methods of Analysis, 18th ed. Maryland, USA: AOAC INTERNATIONAL.

Badawy,W., & Smetanska, I. (2020).Utilization of Olive Pomace as a Source of Bioactive Compounds in Quality Improving of Toast Bread. *Egyptian Journal of Food Science*, 48(1): 27-40.

Balli, D., Cecchi, L., Innocenti, M., Bellumori, M., & Mulinacci, N. (2021). Food By-Products Valorisation: Grape Pomace and Olive Pomace (pâté) as Sources of Phenolic Compounds and Fiber for Enrichment of Tagliatelle Pasta. *Food Chemistry*, 355, 129642.

Bandonienė,D., Murkovic, M., Pfannhauser, W., Venskutonis, P., & Gruzdienė, D. (2002). Detection and Activity Evaluation of Radical Scavenging Compounds by using DPPH Free Radical and On-Line HPLC-DPPH Methods. *European Food Research and Technology,* 214(2): 143-147.

Barba,F. J., Zhu, Z., Koubaa, M., Sant'Ana, A. S., & Orlien, V. (2016). Green Alternative Methods for the Extraction of Antioxidant Bioactive Compounds from Winery Wastes and By-products: A review. *Trends in Food Science & Technology,* 49: 96-109.

Cassano, A., De Luca, G., Conidi, C., & Drioli, E. (2017). Effect of Polyphenols-Membrane Interactions on the Performance of Membrane-Based Processes.A review. *Coordination Chemistry Reviews*, 351: 45-75.

Cecchi, L., Schuster, N., Flynn, D., Bechtel, R., Bellumori, M., Innocenti, M., ... & Guinard, J. X. (2019). Sensory Profiling and Consumer Acceptance of Pasta, Bread, and Granola Bar Fortified with Dried Olive Pomace (Pâté): a Byproduct from Virgin Olive Oil Production.*Journal of food science*, 84(10), 2995-3008.

Chanioti,S., & Tzia, C. (2017). Optimization of Ultrasound-Assisted Extraction of Oil from Olive Pomace using Response Surface Technology: Oil Recovery, Unsaponifiable Matter, Total Phenol Content and Antioxidant Activity. *LWT-Food Science and Technology,* 79: 178-189.

Cho,W. Y., Kim, D. H., Lee, H. J., Yeon, S. J., & Lee, C. H. (2020). Quality Characteristic and Antioxidant Activity of Yogurt Containing Olive Leaf Hot Water Extract. *CyTA-Journal of Food*, 18(1): 43-50.

Chouchouli,V., Kalogeropoulos, N., Konteles, S. J., Karvela, E., Makris, D. P., & Karathanos, V. T. (2013). Fortification of Yoghurts with Grape (Vitis vinifera) Seed Extracts. *LWT-Food Science and Technology,* 53(2): 522-529.

Conterno, L., Martinelli, F., Tamburini, M., Fava, F., Mancini, A., Sordo, M., ... & Tuohy, K. (2019). Measuring the Impact of Olive Pomace Enriched Biscuits on the Gut Microbiota and its Metabolic Activity in Mildly Hypercholesterolaemic Subjects.*European journal of nutrition*, 58(1): 63-81.

de Jesus, S. S., & Maciel Filho, R. (2020). Recent Advances in Lipid Extraction using Green Solvents.*Renewable and Sustainable Energy Reviews*, 133, 110289.

De la Torre-Carbot, K., Jauregui, O., Gimeno, E., Castellote, A. I., Lamuela-Raventós, R. M., & López-Sabater, M. C. (2005). Characterization and Quantification of Phenolic Compounds in Olive Oils by Solid-Phase Extraction, HPLC-DAD, and HPLC-MS/MS. *Journal of agricultural and food chemistry*, 53(11): 4331-4340.

Department of Statistics, Agriculture; Jordan. (2017). http://dosweb.dos.gov.jo/products/agricultural-statistics-2017/

Difonzo,G., Troilo, M., Squeo, G., Pasqualone, A., & 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.

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). (2011), Scientific Opinion on the Substantiation of Health Claims Related to Polyphenols in Olive and Protection of LDL Particles from Oxidative Damage (ID 1333, 1638, 1639, 1696, 2865), Maintenance of Normal Blood HDL Cholesterol Concentrations (ID 1639), Maintenance of Normal Blood Pressure (ID 3781),"Anti-Inflammatory Properties"(ID 1882),"Contributes to the Upper Respiratory Tract Health"(ID 3468),"Can Help to Maintain a Normal Function of Gastrointestinal Tract"(3779), and "Contributes to Body Defences ....*EFSA journal,* 9(4), 2033

Elewa,N. A. H., Degheidi, M. A., Zedan, M. A., & Malim, M. A. (2009). Synergistic Effects of Inulin and Cellulose in UF-Probiotic White Soft Cheese. *Egyptian Journal of Dairy Science,* 37(1): 85-100.

FAO (2015) Milk facts. http://www.fao.org/resources/infographics/infographics-details/en/c/273893/. Accessed 7 Aug 2016.

Fitzgerald, D. J., Stratford, M., & Narbad, A. (2003). Analysis of the Inhibition of Food Spoilage Yeasts by Vanillin. *International journal of food microbiology,* 86(1-2): 113-122.

Fleming, H. P., Walter Jr, W. M., & Etchells, J. (1973). Antimicrobial Properties of Oleuropein and Products of its Hydrolysis from Green Olives. *Applied microbiology,* 26(5): 777-782.

Galanakis,C. M. (2011). Olive Fruit Dietary Fiber: Components, Recovery and Applications. *Trends in Food Science & Technology,* 22(4): 175-184.

Gómez-Cruz,I., Cara, C., Romero, I., Castro, E., & Gullón, B. (2020).Valorisation of Exhausted Olive Pomace by An Eco-Friendly Solvent Extraction Process of Natural Antioxidants.*Antioxidants,* 9(10), 1010.

Gómez-Muñoz,B., Hatch, D. J., Bol, R., & García-Ruiz, R. (2012). The Compost of Olive Mill Pomace: From a Waste to a Resource-Environmental Benefit of its Application in Olive Oil Groves. *Sustainable Development-Authoritative and Leading Edge Content for Environmental Management*, 459-484.

Gorelik, S., Kanner, J., Schurr, D., & Kohen, R. (2013). A Rational Approach to Prevent Postprandial Modification of LDL by Dietary Polyphenols. *Journal of Functional Foods*, 5(1): 163-169.

Gülçin, İ. (2006). Antioxidant Activity of Caffeic acid (3, 4-dihydroxycinnamic acid). *Toxicology*, 217 :(2-3), 213-220.

Gullon,B., Eibes, G., Moreira, M. T., Herrera, R., Labidi, J., & Gullon, P. (2018). Yerba mate waste: A Sustainable Resource of Antioxidant Compounds. *Industrial Crops and Products*, 113: 398-405.

Haddad, M.A., & Yamani, M. I. (2017). Microbiological Quality of Soft White Cheese Produced Traditionally in Jordan. *J Food Process Technol*, 8(12): 706-712.

Haddadin, J. S. (2005). Kinetic Studies and Sensorial Analysis of Lactic Acid Bacteria Isolated from White Cheese Made from Sheep Raw Milk. *Pakistan Journal of Nutrition,* 4(2): 78-84.

Haddadin, M. S., Haddadin, J., Arabiyat, O. I., & Hattar, B. (2009).Biological Conversion of Olive Pomace into Compost by using Trichoderma Harzianum and Phanerochaete Chrysosporium.*Bioresource technology*, 100(20): 4773-4782.

Harkat-Madouri, L., Asma, B., Madani, K., Said, Z. B. O. S., Rigou, P., Grenier, D., ... & Boulekbache-Makhlouf, L. (2015). Chemical Composition, Antibacterial and Antioxidant Activities of Essential Oil of Eucalyptus Globulus from Algeria. *Industrial Crops and Products,* 78: 148-153.

Hassim, N., Markom, M., Anuar, N., & Baharum, S. N. (2014). Solvent Selection in Extraction of Essential Oil and Bioactive Compounds from Polygonum Minus. *Journal of Applied Sciences*, 14(13): 1440-1444.

Humeid, M. A., & Tukan, S. (1986). Toward Improving the Traditional Method of Producing Boiled Brined White Cheese (Nablusi Cheese). *Dirasat* (Jordan).

Humeid,M. A., Tukan, S. K., & Yamani, M. I. (1990).In-bag Steaming of White Brined Cheese as a Method for Preservation. *Milchwissenschaft,* 45(8): 513-516.

Innosa, D., Ianni, A., Faccia, M., Martino, C., Grotta, L., Saletti, M. A.,& Martino, G. (2020). Physical, Nutritional, and Sensory Properties of Cheese Obtained from Goats Fed a Dietary Supplementation with Olive Leaves.*Animals,* 10(12), 2238.

International Organization for Standardization 21527-1: 2008 (E): *Microbiology of Food and Animal Feeding Stuffs - Horizontal Method for the Enumeration of Yeasts and Moulds - Part 1*: Colony Count Technique in Products with Water Activity Greater than 0.95. Switzerland.

International Organization for Standardization 4833-1: 2013 (E): *Horizontal Method for the Enumeration of Microorganisms*, Colony - Count Technique at 30 ° C . Switzerland.

International Organization for Standardization 6888-1: (1999). *Microbiology of Food and Animal Feeding Stuffs - Horizontal Method for the Enumeration of Coagulase*

Positive Staphylococci (Staphylococcus aureus and other species) -Part 1: Technique using Baird - Parker Agar Medium.Switzerland.

International Organization for Standardization: (2003). *Sensory Analysis-Guidelines for the Use of Quantitative Response Scales*. ISO

International Organization of Standardization 6887-1: (1999). *Microbiology of Food and Animal Feeding Stuffs - Preparation* of Test Samples, Initial Suspension and Decimal Dilutions for Microbiological Examination - Part 1: General Rules for the Preparation of the Initial Suspension and Decimal Dilutions. Switzerland.

Irakli, M., Chatzopoulou, P., & Ekateriniadou, L. (2018). Optimization of Ultrasound-Assisted Extraction of Phenolic Compounds: Oleuropein, Phenolic acids, Phenolic Alcohols and Flavonoids from Olive Leaves and Evaluation of its Antioxidant Activities. *Industrial Crops and Products*, 124: 382-388.

Jakobek, L., & Matić, P. (2019). Non-Covalent Dietary Fiber-Polyphenol Interactions and their Influence on Polyphenol Bioaccessibility. *Trends in Food Science & Technology*, 83: 235-247.

Kim,Y. K., Nam, M. S., & Bae, H. C. (2017). Characteristics of Gouda Cheese Supplemented with Chili Pepper Extract Microcapsules.*Korean Journal for Food Science of Animal Resources,* 37(6), 833.

Klen, T. J., & Vodopivec, B. M. (2012). The Fate of Olive Fruit Phenols during Commercial Olive Oil Processing: Traditional Press versus Continuous Two-and Three-Phase Centrifuge. *LWT-Food Science and Technology*, 49(2): 267-274.

Leouifoudi,I., Zyad, A., Amechrouq, A., Oukerrou, M. A., Mouse, H. A., & Mbarki, M. (2014). Identification and Characterisation of Phenolic Compounds Extracted from Moroccan Olive Mill Wastewater. *Food Science and Technology,* 34: 249-257.

Lin, S., Chi, W., Hu, J., Pan, Q., Zheng, B., & Zeng, S. (2017). Sensory and Nutritional Properties of Chinese Olive Pomace Based High Fibre Biscuit. *Emirates Journal of Food and Agriculture*, 495-501.

Lindmark Månsson, H. (2008). Fatty Acids in Bovine Milk Fat. *Food & nutrition research,* 52(1), 1821.

Lindqvist,R., Sylvén, S., & Vågsholm, I. (2002). Quantitative Microbial Risk Assessment Exemplified by *Staphylococcus aureus* in Unripened Cheese Made from Raw Milk. *International Journal of Food Microbiology*, 78(1-2): 155-170.

Liu,X., Dong, M., Chen, X., Jiang, M., Lv, X., & Yan, G. (2007).Antioxidant Activity and Phenolics of an Endophytic Xylaria sp. from Ginkgo Biloba.*Food chemistry*, 105(2): 548-554.

Mattas,K., & Tsakiridou, E. (2017). 35 The Functional Olive Oil Market: Marketing Prospects and Opportunities.*Olives and Olive Oil as Functional Foods*, 647.

Mehyar, G. F., Al-Ismail, K., Han, J. H., & Chee, G. W. (2012). Characterization of Edible Coatings Consisting of Pea Starch, Whey Protein Isolate, and Carnauba Wax and Their Effects on Oil Rancidity and Sensory Properties of Walnuts and Pine Nuts. *Journal of food science*, 77(2): E52-E59.

Nunes, M. A., Palmeira, J. D., Melo, D., Machado, S., Lobo, J. C., Costa, A. S. G., ... & Oliveira, M. B. P. P. (2021). Chemical Composition and Antimicrobial Activity of a New Olive Pomace Functional Ingredient. *Pharmaceuticals,* 14(9), 913.

Nunes,M. A., Pimentel, F. B., Costa, A. S., Alves, R. C., & Oliveira, M. B. P. (2016). Olive By-products for Functional and Food Applications: Challenging Opportunities to face Environmental Constraints.*Innovative Food Science & Emerging Technologies*, 35: 139-148.

Paixao,N., Perestrelo, R., Marques, J. C., & Câmara, J. S. (2007). Relationship between Antioxidant Capacity and Total Phenolic Content of Red Rosé and White Wines. *Food Chemistry,* 105(1): 204-214.

Panzella, L., Moccia, F., Nasti, R., Marzorati, S., Verotta, L., & Napolitano, A. (2020). Bioactive Phenolic Compounds from Agri-Food Wastes: An update on Green and Sustainable Extraction Methodologies. *Frontiers in nutrition*, 7, 60.

Portarena,S., Baldacchini, C., & Brugnoli, E. (2017). Geographical Discrimination of Extra-Virgin Olive Oils from the Italian Coasts by Combining Stable Isotope Data and Carotenoid Content within a Multivariate Analysis. *Food Chemistry*, 215, 1-6.

Raharjo,S., Sofos, J. N., & Schmidt, G. R. (1993). Solid-Phase Acid Extraction Improves Thiobarbituric Acid Method to Determine Lipid Oxidation. *Journal of Food Science,* 58(4): 921-924.

Reboredo-Rodríguez,P., Figueiredo-González, M., González-Barreiro, C., Simal-Gándara, J., Salvador, M. D., Cancho-Grande, B., & Fregapane, G. (2017).State of the Art on Functional Virgin Olive Oils Enriched with Bioactive Compounds and their Properties. *International Journal of Molecular Sciences*, 18(3), 668.

Ribeiro, T. B., Bonifácio-Lopes, T., Morais, P., Miranda, A., Nunes, J., Vicente, A. A., & Pintado, M. (2021). Incorporation of Olive Pomace Ingredients into Yoghurts as a Source of Fibre and Hydroxytyrosol: Antioxidant Activity and Stability throughout Gastrointestinal Digestion. *Journal of Food Engineering,* 297, 110476.

Ribeiro, T. B., Oliveira, A., Coelho, M., Veiga, M., Costa, E. M., Silva, S., ... & Pintado, M. (2022). Are Olive Pomace Powders a Safe Source of Bioactives and Nutrients? *Journal of the Science of Food and Agriculture*, 101(5): 1963-1978.

Rodríguez-Rojo, S., Visentin, A., Maestri, D., & Cocero, M. J. (2012). Assisted Extraction of Rosemary Antioxidants with Green Solvents. *Journal of Food Engineering,* 109(1): 98-103.

Roselló-Soto, E., Koubaa, M., Moubarik, A., Lopes, R. P., Saraiva, J. A., Boussetta, N., ... & Barba, F. J. (2015). Emerging Opportunities for the Effective Valorization of Wastes and By-products Generated during Olive Oil Production Process: Non-Conventional Methods for the Recovery of High-Added Value Compounds. *Trends in Food Science & Technology,* 45(2): 296-310.

Şahin,S., & Şamlı, R. (2013).Optimization of Olive Leaf Extract Obtained by Ultrasound-Assisted Extraction with Response Surface Methodology. *Ultrasonics Sonochemistry,* 20(1): 595-602.

Salah,M. B., Abdelmelek, H., & Abderraba, M. (2012).Study of Phenolic Composition and Biological Activities Assessment of Olive Leaves from Different Varieties Grown in Tunisia.*Med chem*, 2(5): 107-111.

Salomone,R., & Ioppolo, G. (2012). Environmental Impacts of Olive Oil Production: a Life Cycle Assessment case study in the province of Messina (Sicily). *Journal of cleaner production,* 28: 88-100.

Simonato,B., Trevisan, S., Tolve, R., Favati, F., & Pasini, G. (2019). Pasta Fortification with Olive Pomace: Effects on the Technological Characteristics and Nutritional Properties.*LWT*, 114, 108368.

Simsek,M., & Süfer, Ö. (2022). Olive Pomace from Olive Oil Processing as Partial Flour Substitute in Breadsticks: Bioactive, Textural, Sensorial and Nutritional Properties.*Journal of Food Processing and Preservation*, 46(6), e15705.

Soler-Rivas, C., Espín, J. C., & Wichers, H. J. (2000). Oleuropein and Related Compounds. *Journal of the Science of Food and Agriculture*, 80(7): 1013-1023.

Spinelli, S., Conte, A., Lecce, L., Incoronato, A. L., & Del Nobile, M. A. (2015). Microencapsulated Propolis to Enhance the Antioxidant Properties of Fresh Fish Burgers. *Journal of Food Process Engineering*, 38(6): 527-535.

Tang, J. (2003). Drum Drying D. *Encyclopedia of Agricultural, Food, and Biological Engineering* (Print), 211.

Uribe, E., Lemus-Mondaca, R., Pasten, A., Astudillo, S., Vega-Gálvez, A., Puente-Díaz, L., & Di Scala, K. (2014). Dehydrated Olive-Waste Cake as a Source of High Value-Added Bioproduct: Drying Kinetics,

Physicochemical Properties, and Bioactive Compounds.*Chilean journal of agricultural research,* 74(3), 293-301.

Van Soest,P.V., Robertson, J. B., & Lewis, B. A. (1991).Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition.*Journal of dairy science,* 74(10), 3583-3597.

Vitali Čepo, D., Radić, K., Jurmanović, S., Jug, M., Grdić Rajković, M., Pedisić, S., ... & Albahari, P. (2018). Valorization of Olive Pomace-Based Nutraceuticals as Antioxidants in Chemical, Food, and Biological Models. *Molecules*, 23(8): 2070.

Vlyssides, A. G., Loizidou, M., Gimouhopoulos, K., & Zorpas, A. (1998). Olive Oil Processing Wastes Production and their Characteristics in Relation to Olive Oil Extraction Methods.

Winkelhausen, E., Pospiech, R., & Laufenberg, G. (2005). Antifungal Activity of Phenolic Compounds Extracted from Dried Olive Pomace. *Bulletin of the Chemists and Technologists of Macedonia,* 24(1): 41-46.

Xue, J., Davidson, P. M., & Zhong, Q. (2013). Thymol Nanoemulsified by Whey Protein-Maltodextrin Conjugates: The Enhanced Emulsifying Capacity and Antilisterial Properties in Milk by Propylene Glycol. *Journal of agricultural and food chemistry,* 61(51): 12720-12726.

Yakhlef, W., Arhab, R., Romero, C., Brenes, M., de Castro, A., & Medina, E. (2018). Phenolic Composition and Antimicrobial Activity of Algerian Olive Products and By-products. *LWT*, 93: 323-328.

Zhao,H., Avena-Bustillos, R. J., & Wang, S. C. (2022).Extraction, Purification and In Vitro Antioxidant Activity Evaluation of Phenolic Compounds in California Olive Pomace. *Foods,* 11(2), 174.

الانشطة المضادة للميكروبات وللأكسدة لمستخلص جفت الزيتون في الجبنة البيضاء المبسترة

هاجر م. فرقوس[1]، غدير فوزي مهيار [1]*وسلام أ. إبراهيم[2]

[1] قسم التغذية والتصنيع الغذائي، كلية الزراعة، الجامعة الأردنية، الأردن.
[2]جامعة ولاية كارولينا الشمالية الزراعية والتقنية، الولايات المتحدة الأمريكية

تاريخ استلام البحث: 2022/9/20 وتاريخ قبوله: 2023/2/26.

ملخص

تم جمع ثفل الزيتون من معاصر الزيتون في ديسمبر 2021. أظهر التحليل التقريبي أن المكونات الرئيسية للثفل هي الألياف (60.85٪) والدهن (11.91٪). أعطى مستخلص ثفل الزيتون بواسطة الميثانول إنتاجية أعلى من محتوى الفينول وقدرة أعلى على ازالة الجذور الحرة من مستخلص الإيثانول، ولكن تم استخدام الأخير نظرًا لانخفاض سمنته عند استخدامه في الاغذية. يحتوي المُستخلص على تركيزات عالية من المركبات الفينولية وخاصة caffeic acid، vanillin، p-coumaric، syringic acid والتي تمثل حوالي 80٪ من إجمالي محتوى الفينول. ان استخدام (0.08٪) من مستخلص ثفل الزيتون (OPE) في الجبن الأبيض المبستر، قد حد من نمو الكائنات الحية المجهرية الهوائية والمحبة للحرارة المتوسطة إلى 3.62 لوغ وحدة مكونة للمستعمرة/ غم بعد 30 يومًا من التخزين عند C5° بالمقارنة مع الشاهد (4.04 لوغ وحدة مكونة للمستعمرة/ غم) بعد نفس فترة التخزين. كانت القيم المقابلة لبكتيريا حمض اللاكتيك 3.38 لوغ وحدة مكونة للمستعمرة/ غم و 3.79 لوغ وحدة مكونة للمستعمرة/ غم على التوالي. تم القضاء على الخمائر والعفن من الجبن لمدة 14 يومًا في جميع تراكيز OPE المفحوصة (0.01٪ إلى 0.08٪). ان تحصين الجبن بال OPE قد خفف من زيادة الحموضة المعايرة في الجبن أثناء التخزين، ولكن للجبن المحصن سمات حسية مماثلة لجبن التحكم. يمكن الاستنتاج أن تحصين الجبن الأبيض المبستر بـOPE يحسن الصفات الكيميائية والميكروبية للجبن.

الكلمات الدالة: ثفل الزيتون، الجبن الأبيض المبستر، إجمالي البوليفينول، مضادات الأكسدة، مضادات الميكروبات.

* الباحث المعتمد للمراسلة: g.mehyar@ju.edu.jo