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Wonderful Pomegranate (*Punica granatum* L.) Juice Wastes Extract as a Food Preservative

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THIS INVESTIGATION was carried out to prepare lyophilized extract from the pomegranate juice extracting wastes, determine its content and type of polyphenols and its use as a natural an antioxidant in stabilizing sunflower oil oxidation, an antimicrobial against some pathogenic bacteria and a preservative to extend the shelf life of chilled chicken patties during cold storage. The results showed that, the yield of the lyophilisate was 24.91 ± 1.29% of the dried pomegranate juice processing wastes. The content of total polyphenols increased from 10.51 mg/g in crude extract of wastes to 852.43 mg/g after concentration then lowered to 783.2 mg/g due to grinding and packaging after lyophlization. Punicalagin A (120 mg/g), punicalagin B (16mg/g), gallic acid (2.11 mg/g) and ellagic acid (2 mg/g) were identified in the lyophilized extract.The highest inhibitory effect of lyophilisate was125 ppm for both *S. aureus* and *K. pneumonia* ,250 ppm for *E. coli* and 500 ppm for both *S. senftenberg* and *B. subtilus*.The control sample of sunflower oil exhibited the highest value of peroxide value, conjugated dienes (CD) and conjugated trienes (CT) inaddition to thiobarbituric acid (TBA) followed by those containing 200, 500, BHT (200 ppm) and 750 ppm of the lyophilized extract. Also addition of the lyophilizate at 1500 and 2000 ppm to chicken patties extended its shelf life to 24 days at 4 ^{o}C with slight changes in its pH value, microbial load, TBA and phenolic content.

Introduction

Pomegranate juice extractingwastes (peels, rind and pith) are rich source of tannins and other phenolic compounds. It can offer a practical and an economic source of potent natural antimicrobial and antioxidants that could replace synthetic food preservatives. Wang et al., (2004) reported that methanol is considered an effective solvent to extract broad range of polyphenols from pomegranate marc, rind and peels. It is also cheap and easily accessible with aqueous solution. The yield of the extracted polyphenols from the total polyphenols content of the dried pomegranate peels powder was the highest when extracted by methanol (~47%) followed by , ethanol (~18%), acetone (4%), and ethyl acetate (1%), respectively. (Singh *et al*., 2000 and Wang et al., 2010).

Methanolic extract of such wastes were found to be effective against *Staphylococcus aureus, Eschericia coli, Klebsiella pneumonia, Proteus vulgaris and Salmonella typhimirium.* Punicalagins isolated from pomegranate fruit peels extract exhibited antifungal activity against *Candida albicans, Pencilliumcitrinum* and *Aspergillus ochraceus* (Vasconcelos et al., 2003 and Dahham et al., 2010).Generally, the antimicrobial inactivation thresholds depend on the specific targets of these substances. These targets may be cell wall, cell membrane, metabolic enzymes, protein synthesis, and genetic system of microorganisms (Raybaudi-Massilia et al., 2009). Tannins form complexes with metal ion and decrease from their availability to bacteria and subsequently, the metallo-enzymes activity in microbial cell wall be affected (Raybaudi-Massilia et al., 2009, Khan and Sonali, 2011). Study of Anibal et al., (2013) on the effect of the ethanolic crude pomegranate peel extract on the morphological and structure of *Candida* spp by scanning electron microscopy (ESM) showed an irregular membrane, hyphe formation vacuoles and an increase in thickness of the cell wall of the *Candida* spp treated with this extract.

Methanolic extract of pomegranate peel considers a natural potent antioxidant as

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demonstrated by Iqbal et al. (2008).Two tannins neamely, ellagic acid and punicalagin play an important role in the antioxidant activity of this extract. Ellagic acid can react with free radicals due to its ability to chelate metal ions. Punicalagin is able to inhibit lipid peroxidation by providing electrons able to eliminate the resulted free radicals from lipid oxidation (Adams et al., 2006). This extract can replace the synthetic antioxidants such as butylated hydroxy anisol (BHA) and butylated hydroxy tolune (BHT). Such antioxidants are suspected to be carcinogenic (Singh et al., 2002).

This investigation aimed to prepare lyophilized methanol extract from the pomegranate juice extracting wastes to use as a natural an antioxidant in stabilizing sunflower oil and an antimicrobial against selected five strains of pathogenic bacteria in addition to as a preservative in extending the shelf life of chilled chicken patties during cold storage. The isolation, identification and determination of the major polyphenols in such extract were also determined using high pressure liquid chromatography (HPLC).

Materials and Methods

Materials

Ten Kg of Wonderful pomegranate cultivar(*Punica granatum* L.) fruit were purchased from a commercial fruits and vegetables market in Alexandria city , Egypt in August 2015. The fruits were transported and stored at 10 ^{o}C after discarding the injured fruits in the Postharvest Technology Center , Faculty of Agriculture, Alexandria University, Egypt.Five Kg of refined-bleached -deodorized (RBD)sunflower oil free from antioxidant additive was obtained from the Extracted Oil and Derivatives Company , Alexandria, Egypt.Fresh boneless chicken breast meat (three Kg) were purchased from local market, Alexandria, Egypt.Clean reagent brown glass bottles ,250 ml capacity, with waxed tin plate screw caps were obtained from the Egyptian Company for Medicinal Container, Alexandria, Egypt. Transparent colourless polyethylene jars and low density polyethylene bags of 450 g capacity were obtained from the National Plastic Company, Alexandria, Egypt.

Five bacterial strains known to be pathogenic to humans ; *Bacillus subtilis* DB 100 *host; Escherichia coli* BA 12296; *Klebsiell apneumoniae* ATCC12296*; Salmonella senftenberg* ATCC 8400; *Staphylococcus aureus* NCTC 10788 were obtained from the City of Scientific Research and Technological Applications, Borg Alarab, Alexandria, Egypt. The bacterial strains were cultured on nutrient agar medium.

Analytical standards of punicalagin A and B , gallic and ellagic acids, both □-amylase and amyloglucosidase , polyvinyl pyrolidin polymer, butylated hydroxy tolune (BHT), Folin-Ciocalteu·s phenol reagent, cateckin,tannic acid and vanillin reagent were obtained from Sigma-Aldrich Company (St. Louis, Missouri, USA) .HPLC grade O- phosphoric acid (85%), methanol and acetonitrile were bought fromFisher Scientific Inc. (New Jersey, USA).The water used in HPLC analysis was deionized and filtered through a 0.45µm type HA membrane filter. Other chemicals were of analytical grade.Syringe filters made of nitrocellulose (Millipore, Bedford, USA) or polytetrafluro ethylene (Sartorious, Goettingen, Germany) with pore size of 0.45 µm were used to filter aqueous or organic extracts.

Methods

Technological methods

The washed pomegranate fruits were first cut into four pieces to remove their peels and skin covering their arils, then juice of arils was extracted using an electric mixer for 30 sec. and filtered through muslin cloth to remove pith. The obtained wastes of the extracted juice (peels, arils, skin and pith) were sorted to remove foreign matters and collected together before drying under vacuum at 60^{o} C for 48 hr using lab vacuum oven (Model 3618, USA). The dried products were grounded to pass through 40 mesh sieve then extracted in dark (to avoid photo oxidation) at 1200 rpm with an electric shaker (JSSI-100T, South, Korea) using 95% methanol at a ratio of 1:4 (w/v) powder to solvent at room temperature (23±2 ^{o} C) as described by Qu et al. (2012). The methanol extract was separated from the residue by vacuum filtration through Whatman No 1 filter paper. The filtrate was concentrated by using a rotary evaporator (Heidolph 2000, Germany) at 64^{o}C, then lyophilized at -80 ^{o}C , 18 M torr using a laboratory scale freeze drier (Buchi-B290, Flawil, Switzerland), packed in brown glass bottles and stored at 4^{o} C until used. Yield of the lyophilized pomegranate peels extract was calculated using the following equation:

The extracted yield (%) = (the weight of the dried extract / the weight of the dried wastes) X100

The refined blanched deodorized (RBD) free from antioxidants additives sunflower oil was distributed in five of 250 ml capacity brown

glass bottles. The lyophilized polyphenol crude extract of pomegranate juice extracting wastes was added to three of an individual sunflower oil bottles at a concentrations of 200, 500 and 750 ppm .Butylated hydroxy toluene (BHT) at a concentration of 200 ppm was added to one of the bottles, and the last bottle was (free of antioxidants) , for comparison. All the tested oil bottles were stored in an incubator (Binder B28 02-32304,United States) at 65 ° C and analyzed each four days.

Chicken patties were prepared by mincingfresh boneless chicken breast meat twice using meat mincer, then mixing with 1% sodium chloride anddividing into six equal parts. To the first part, 100 mg of BHT per 100 g meat was added after dissolving in 5ml RBD sunflower oil. The same quantity of sunflower oil was added to other parts. The following concentrations: 500, 1000, 1500 and 2000 ppm of lyophilized polyphenol crude extract of pomegranate juice extracting wastes were added to each of the parts 2,3,4 and 5 ,respectively. The six part was used as a control, without antioxidant.The volume of lyophilized polyphenol crude extract of pomegranate juice extraction wastes was replaced with distilled water in both control and BHT containing parts. Each part was thoroughly hand mixed and formed into 100 g patties. The patties were kept in foam plates after wrapping with low density polyethylene sheets. The foam plates were packed inside polyethylene bags, stored at 4° C and analyzed each 4 days for pH, TBA, total phenolic content and total bacterial count until 24 days.

Chemical methods

Total phenolic (TP) contentwas assayed byFolin –Ciocalteu reagent with tannic acid as a standard at a wave length of 765 nm using UV-Vis. Spectrophotometer ,(Ozgen *et al*., 2008)**.** pH values of the chicken patties was measured after mixing 10 grams of the patties with 50 ml distilled water using a digital Toledo Mp 230 pH meter.

The lyophilized polyphenols crude extract was prepared for HPLC analysis by re-extraction with 95% methanol at a ratio of 50: 1 (v/w) solvent to powder at room temperature (23 ± 2 ° C) with stirring at 1200 rpm for 90 min, filtered through a Whatman No.1 filter paper. The residues were re-extracted with the same volume of methanol. The resulted methanol extracts were pooled and filtered through 0.45 µm Millipore filter (Gelman, Laboratory, MI).Stock solutions of punicalagin (1000mg/L), gallic acid (500 mg/L) and ellagic acid (200 mg/L) were prepared in water : methanol (1:1v/v) solvent . The stock solutions was filtered through 0.45 µm Millipore filter (Gelman, Laboratory, MI) and further diluted to 20 fold with water: methanol (1:1 v/v) solvents prior to HPLC analysis. HPLC separation of the major polyphenol constituents of pomegranate wastes lyophilized extract was done as mentioned by Cam et al., (2010) using HPLC, Agilent1200 series liquid chromatography, (Agilent Technologies, CA , USA) on a Zorbox C 18 column (250X 4.6 mm particle size 5 µm, Agilent), 10 µL volume for injection standards and sample, water –acetic acid (98:2 v/v) solvent A, and methanol (solvent B) as mobile phases at a flow rate of 1 ml/min. The elution program was as follows: 5% B for 5 min, 5 to 70% B for 25 min and 70 to 5% B for 10 min. The column temperature was maintained at 35° C and the detection was monitored at 254, 280 nm and 378 nm. The amounts of punicalagin and their derivatives were calculated from chromatograms recorded at 378 nm, while ellagic acid and gallic acid, at a wave length of 254 and 280 nm respectively. UV spectra of components were taken continuously between 200 to 400 nm, through the elution in order to identify and detect components and peaks purity.

Peroxide value (PV) as meq O_2/ Kg oil , conjugated dienes (CD) at a wave length of 232nm., conjugated trienes (CT) at a wave length of 268 nm and thiobarbituric acid (TBA) value as mg malonaldehyde/ Kg oil were estimated in stabilized sunflower oil using the AOCS standard methods (2013).

Microbiological methods

Antibacterial activitywas estimated by using the agar well diffusion method (Perez *et al*., 1995). The nutrient agar plates were prepared and spread with 250 µl(1 x 10^8cfu/ml) of the pathogenic cultures. Wells of 8 mm diameter were pored using sterile borer then were loaded with 100 ul of the tested sample or distilled water as control .The plates were incubated at 37 ° C overnight. The diameter (mm) of the zone of inhibition of the loaded pomegranate juice and peel extract was measured.

Total mesosphilic bacterial countwas determined as reported by Martin et al. (2008) using standard microbiological pour plate technique,plate count agar media recommended by Martin et al., (2008) and incubation at 35-37 °C for 48 hr.

Statistical analysis

The obtaineddata were analyzed using software version 917 (stats soft, Inc. USA, 2003). Analysis of variance (ANOVA) was performed to determine the differences. Differences among means were considered significant at $p > 0.05$ using Duncans·s multiple difference test (Steel and Torrie, 1980).

Results and Discussion

Polyphenols types and content

The content of polyphenols increased from 10.51 mg/ g in the crude methanol extract to be 852.43 mg/g after concentration then lowered to 783.2 mg/g after lyophlization due to a slight oxidation during grinding and packaging afterlyophlization. The yield of the lyophilisate was 24.91 ± 1.29 % of the dried pomegranate juice processing wastes.

The identification and quantification of both Punicalagin A, punicalagin B, gallic acid and ellagic acids of the lyophilized extract are shown in Table 1 and Fig. 1 (A,B,C). As seen from Fig 1(A), there are 4 peaks , peak 2 with a retention time of 32.15 min represented punicalagin A where, peak 4 with a retention time of 55.96 min was punicalagin B. According to Gil et al. (2000) punicalagin A and punicalagin B interconvert rapidly when in solution. Peaks 1 and 3 with a 30.99 and 33.63 min retention times showed similar UV spectra with punicalagin A. Therefore they were evaluated as punicalagin A derivatives (120.29 mg/g). There retention times were very close to thatof punicalagin A. Generally, the total area of such derivatives plus punicalagin A represented nearly 70 % of the total area of HPLC chromatogram at 378 nm (Fig 1 (A).

Figure 1 (B) shows 7 peaks , peak 2 with 5.87 retention time represented the presence of gallic acid (2.11 mg/g) in the lyophilized extract. Ellagic acid (peak number 6 in Fig. 1 C) with 52 min retention time at 254 nm was found as 1.918 mg/g. Zhou et al. (2008) found that ellagic acid and its derivatives ranged from 4.5 to 10.8 mg/g in pomegranate peel extract according to the type of solvent used for extraction.According to Mphahlele et al. (2016) pomegranate fruits are rich source of bioactive compounds, located mainly in fruit peels and mesocarp. They have several functions such as attract insects, protect against UV-radiation, regulate osmotic pressure, exhibit an astringe effect and inhibit enzymes involved in oxidation reactions.

Antimicrobial activity

The results in Table 2 show the effect of a lyophilized extract of wastes on the diameter of the inhibition zone (DIZ) in mm.against the tested pathogenic bacteria. There was a variation in the DIZ values between cultures according to the applied concentration of the lyophilized pomegranate wastes extract. Generally, the antibacterial effect of the extract increased with increasing its applied concentration. In the case of Gram positive *B. subtilis*, the effective concentration ranged from 500 to 2000 ppmwith DIZ ranged from 10 to 19 mm. The highest inhibitory effect was exhibited by adding 2000 ppm followed by both 1000 and 500 ppm of the extract, respectively. Dahham *et al*., (2010) found that pomegranate methanol extract had more inhibitory effect against *B. subtilis* than pomegranate juice. In contrast to *B. subtilis*, the Gram positive bacteria *S. aureus* was affected by a low concentration of the polyphenols lyophilized extract, 125 ppm.

Among the tested three Gram negative bacteria, the highest antibacterial activity was observed for *K. pneumonia* followed by *E.coli* and *S. senftenberg*. The DIZ values ranged from 9 to 15 mm for *K. pneumonia* when the applied polyphenols extract increased from 125 to 2000 ppm. Nearly the same range of DIZ was obtained for *E.coli* when the concentration of lyophilized extract increased from 250 to 2000 ppm. In case of *S. senftenberg* more concentration of such extract was required to inhibit this culture. Dahham et al., (2010) demonstrated that the pomegranate peels extract showed the highest antibacterial effect against *S. aureus* and *K. pneumonia*.

TABLE 1.The major polyphenols in the lyophilized extract of Wonderful pomegranate juice processing wastes.

Compound	Wave length (nm)	Retention time (min)	Concentration (mg/ g)
Punicalagin A	378	36.26	120.29
Punicalagin B	378	55.96	16.11
Gallic acid	280	5.87	2.11
Ellagic acid	254	52.71	1.92

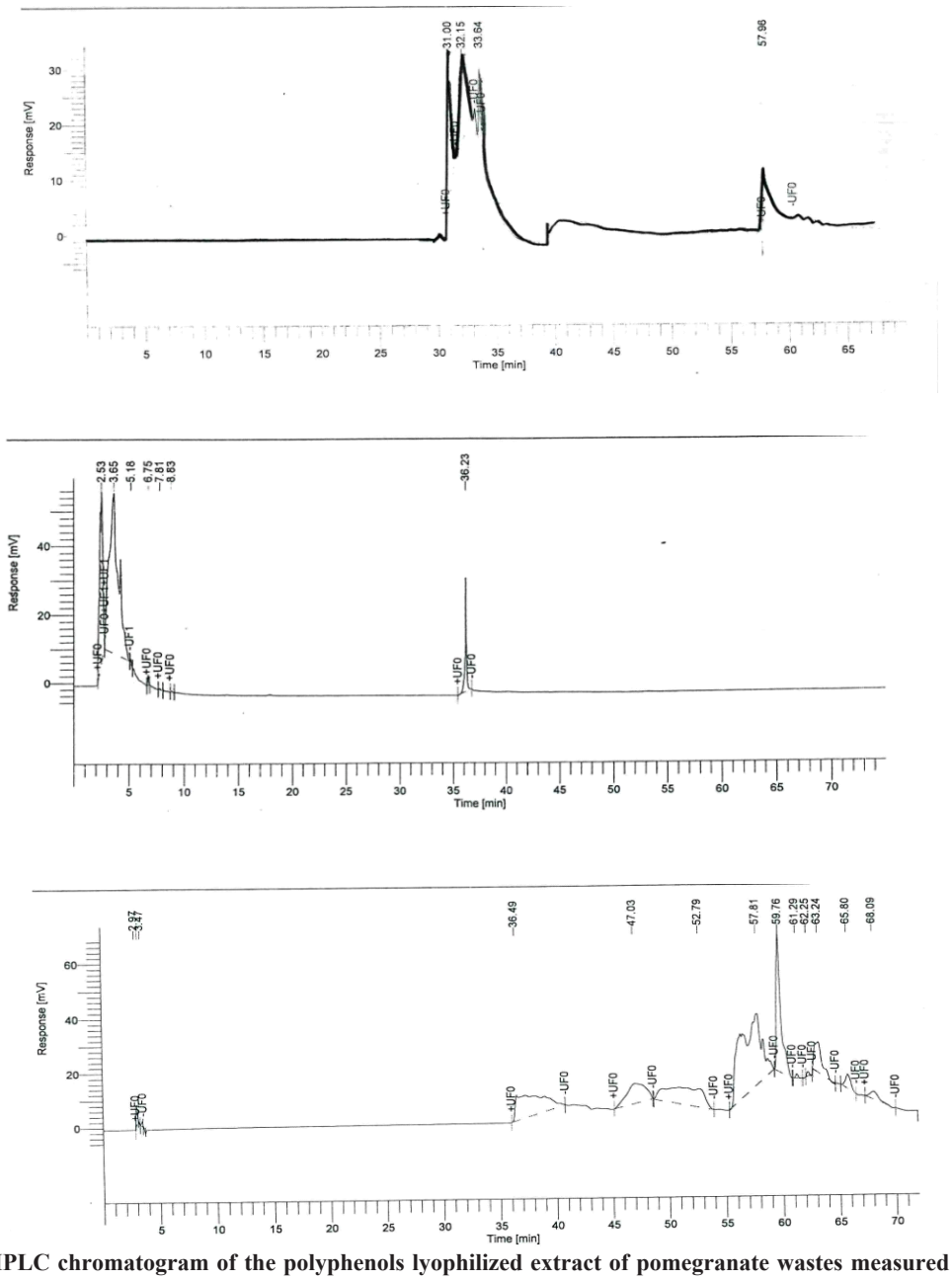

Fig. 1. HPLC chromatogram of the polyphenols lyophilized extract of pomegranate wastes measured at a wave length of 378 (A), 280 (B) and 254 nm (C).

The relation between MIC and the total phenolic content (TP) of the lyophilized wastes extract are presented in Table 3. MIC was 125 ppm (0.1 mg phenolic content /ml as tannic acid equivalent) for both of *S. aureus* and *K. pneumonia*, 250 ppm (0.2 mg polyphenolic content /ml as tannic acid equivalent) for *E. coli* and 500 ppm (0.46mg phenolic content /ml as tannic acid equivalent) for both *S. senftenberg* and *B. subtilus.* According to Naz et al. (2007) pomegranate phenolic compounds, especially gallic acid, exert certain antibacterial effects against *Corynebacterium, Staphylococcus, Streptococcus, B. subtilis, Shigella, Salmonella, Esherichia and vibro* species. Also, Naziri et al. (2012) attributed the stronger effect of pomegranate peels extract on Gram positive bacteria compared to that against Gram negative ones to the differences in cell wall structure in these bacteria. Polyphenols can pass through cell walls, which contain polysaccharides and proteins, and bind to their surfaces causing an interruption to their normal functional and activities.

TABLE 2. Antibacterial effect of lyophilized polyphenols extract of Wonderful pomegranate wastes

Bacterial culture	Concentration of the lyophilized extract				
	125 ppm	250 ppm	500 ppm	1000 ppm	2000 ppm
	Diameter of inhibition zone (DIZ) (mm)				
Bacillus subtilis DB100	NI	NI	10	11	19
Escherichia coli BA 12296	NI	9	10	12	16
Klebsiella pneumonia ATCC12296	9	10	11	12.5	15
Salmonella senftenberg ATCC 8400	NI	NI	10	12	16
Staphylococcus aureus NCTC 10788	10	11	13	14	16

NI means no inhibition

TABLE 3. Minimum inhibitory concentration (MIC) of total phenols of lyophilized Wonderful pomegranate wastes extract

Tested Organism	MIC (ppm)	Total phenols (mg/ml)
Bacillus subtilis	500	0.3916
Escherichia coli	250	0.1958
Klebsiella pneumoniae	125	0.0979
Salmonella senftenberg	500	0.3916
Staphylococcus aureus	125	0.0979

Antioxidant effect

Peroxide value (PV) As shown from Fig. 2 (A), a continuous rise of the PV was detected for all sunflower oil samples with the extend of storage period at 65°C. Formation of hydroperoxides, the primary oxidation products, was behind this increase. The rate of increase was slow in the first 4 days of storage, then increasedrapidly through the next 16 days of storage, to reach a maximum at the 20-th day, then decreased due to the degradation of the primary hydroperoxides to form secondary hydroperoxides. After 24 days of storage at 65 ° C, PV value was in the range of 80 to 120 meq O_2/Kg for lyophilized pomegranate extracts stabilized samples , 97 meq O_2/Kg for BHT stabilized sample and more than 130 meq O_2/Kg for control. Higher PV was observed for the control samples followed in a descending order by SFO-200, SFO-500, SFO-BHT and SFO-750,respectively. A concentration of 750 ppm of the lyophilized extract , as a natural safe antioxidant, showed a stabilizing effect comparable to BHT at 200 ppm, as illustrated in Fig (2, A).

Conjugated diene (CD) and conjugated triene (CT):Figs 3(A and B) show the relative increase in CD and CT of sunflower oil samples, control and those containing the lyophilized pomegranate wastes extract and BHT. The control sample exhibited the highest CD and CT followed by SFO-200, SFO-500, SFO-BHT and SFO-750 ,respectively during all the stages of storage period. Iqbal et al., (2008) stated that CT may be produced by the dehydration of CD hydroperoxides. The rate of CD increase in control sample was tremendous after 8 days of storage. It was quite matching in both SFO-250 and SFO-500 up to 20 days of storage. In contrast, its increase in SFO-750 oil sample through the storage period was low. Same results were noticed for CT content. Generally, the rate of the increase in both of CD and CT in the SFO-750 samplewas obviously lower than the other stabilized oil samples including that containing BHT.

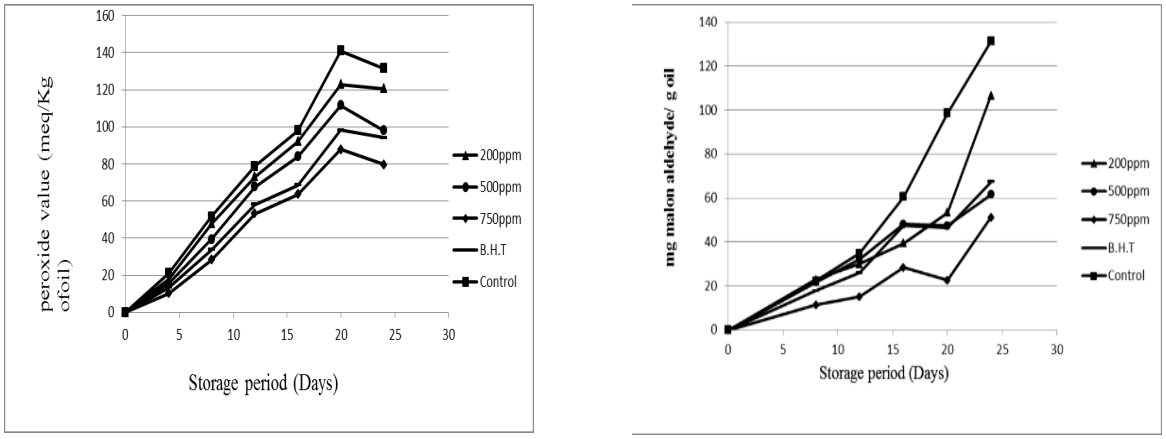

Fig .2. Peroxide value (A) and TBA (B) of sunflower oil under accelerated storage conditions.

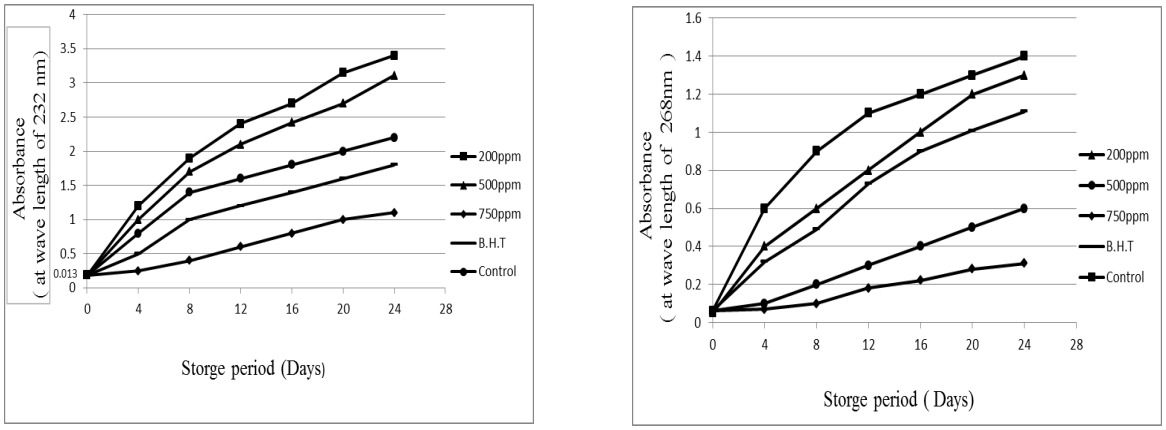

Fig. 3.Conjugated diene (CD) (A) andConjugated triene (CT) (B)of sunflower oil under accelerated storage conditions.

Thiobituric acid (TBA) As seen from Fig (2, B), a gradual increase in TBA was observed for all the SFO- stabilized and unstabilized samples, with extending the storage period under accelerated conditions. Control sample showed the highest TBA values at all the stages of analysis during storage. The initial rate of the TBA formation was slow until the 20-th day of storage then became sharp in the last 4 days of storage of the stabilized SFO. Such changes were noticed after 8 days of storage of control sample.Addition of 750 ppm of the lyophilized extract of pomegranate wastes to SFO exhibited higher stabilization efficiency compared with the other added levels.

Preservation action

Data in Table 4 reveal that the initial pH of the fresh chicken patties ranged between 5.72 and 6.0. pH was decreased significantly with the increase of the added lyophilized extract and extending the storage period. The phenolic acids in the lyophilized extract may be behind the decrease in the pH value of the patties samples containing that extract.Also the acid nature of BHT caused drop in patties pH containing this antioxidant.Hayam et al. (2012) found that cooked meat patties containing pomegranate rind and pomegranate seed extracts had lower pH value than the control samples.

Data in Table 5 indicate that total phenolic content was gradually increased with the increase of the addition of the lyophilized pomegranate wastes extract. It was also significantly higher in patties containing BHT than the control.It was steadily reduced with extending storage period at 4^{o} C.This may be due to the oxidation of the phenolic compounds as well as the binding between polyphenols in the extract with the protein of chicken patties. Such interaction can also influence the cooking quality, time and yield, of such product. Nearly the same observations

were reported by Hayam et al. (2012) for cooked meat patties containing both pomegranate rind and seeds extracts comparing to the control sample.

Comparing with control, addition of the lyophilized extract at different levels and BHT caused significant decrease in the TBA formation in chicken patties during storage at 4° C (Table 6). Addition of 1500 ppm of pomegranate extract to the uncooked chicken patties had nearly similar effect of BHT addition on TBA formation in chicken patties during storage of this product at 4° C. Increasing addition level of the extract to 2000 ppm caused the highest significant decrease of TBA formation during the refrigerated storage (Table 6).

TABLE 4. Effect of polyphenols lyophilized pomegranate wastes extract and BHT on the pH value of chicken patties during storage at 4 °C

Patties type	0	4 days	8 days	12 days	16 days	20 days	24 days	Means
Control	6.08±0.01	6.07±0.05	6.00±0.07	5.94±0.13	5.55±0.13	5.44±0.05	5.22±0.04	5.77 [a]
500 ppm	5.99±0.08	5.96±0.104	6.15±0.11	6.04±0.06	5.56±0.05	5.42±0.06	5.21±0.01	5.73 [b]
1000 ppm	5.93±0.03	5.91±0.04	5.84±0.08	5.61±0.04	5.60±0.03	5.34±0.03	5.19±0.01	5.64 [c]
1500 ppm	5.93±0.03	5.71±0.026	5.64±0.01	5.51±0.02	5.53±0.03	5.41±0.01	5.16±0.03	5.53 [d]
2000 ppm	5.72±0.04	5.67±0.05	5.62±0.03	5.41±0.02	5.51±0.015	5.33±0.02	5.15±0.05	5.51 [d]
BHT	5.78±0.03	5.68±0.02	5.65±0.04	5.48±0.10	5.41±0.023	5.09±0.02	5.09±0.066	5.45 [e]
Means	5.88 [a]	5.86 [a]	5.82 [b]	5.66 [c]	5.53 [d]	5.31 [e]	5.17 [f]	

Different letters in columns and rows indicate significant different values at $P < 0.05$.

TABLE 5. Polyphenols content in chicken patties containing lyophilized pomegranate wastes extract and BHT

Patties type	Total polyphenols content (mg/g as tannic acid equivalent) in chicken patties containing lyophilized pomegranate wastes extract and BHT during storage at 4° C.							
	0	4 days	8 days	12 days	16 days	20 days	24 days	Means
Control	151.84±0.30	148.46±1.41	144.77±1.74	135.84±2.05	130.16±0.36	122.70±1.23	116.08±0.66	135.69 [f]
500 ppm	162.56±2.02	160.38±1.36	155.09±1.24	146.13±3.95	139.55±2.93	138.12±2.10	128.96±2.07	147.26 [d]
1000ppm	172.49±3.19	172.38±1.82	169.16±2.67	158.88±4.81	152.43±8.28	151.65±1.07	140.12±0.97	159.59 [c]
1500pp	190.75±2.19	188.12±3.18	182.23±2.15	171.87±3.64	165.08±4.24	169.64±0.95	160.44±1.58	175.45 [b]
2000ppm	227.37±7.03	221.45±0.90	216.19±9.72	204.94±10.36	194.97±9.73	178.80±2.34	170.54±0.75	202.04 [a]
BHT	159.04±0.90	155.13±1.09	150.96±0.95	139.27±1.077	130.40±1.64	130.14±0.98	121.13±0.96	140.870 [e]
Means	177.34 [a]	174.32 [b]	169.73 [c]	159.49 [d]	152.10 [e]	148.51 [f]	139.54 [g]	

Different letters in columns and rows indicate significant different values at $P < 0.05$.

TABLE 6. Thiobarbituric acid value (TBA) of chicken patties containing lyophilized pomegranate wastes extract and BHT

Patties type	Thiobarbituric acid value (TBA value mg malonaldehyd/g) of chicken patties containing lyophilized pomegranate wastes extract and BHT during storage at 4^{O} C							
	0	4 days	8 days	12 days	16 days	20 days	24 days	Means
Control	0.40±0.02	0.69±0.01	1.11±0.30	1.45±0.017	1.66±0.01	1.48±0.35	1.99±0.04	1.25 ^{a}
500 ppm	0.32±0.02	0.42±0.01	0.61±0.016	0.813±0.05	1.22±0.12	1.28±0.10	1.64±0.04	0.90 ^{b}
1000ppm	0.20±0.20	0.26±0.02	0.40±0.02	0.62±0.015	0.80±0.05	1.27±0.23	1.23±0.01	0.68 ^{c}
1500ppm	0.18±0.10	0.18±0.05	0.19±0.19	0.26±0.04	0.43±0.025	0.64±0.01	0.85±0.035	0.39 ^{d}
2000ppm	0.10±0.18	0.10±0.10	0.11±0.11	0.14±0.046	0.23±0.01	0.41±0.01	0.60±0.05	0.24 ^{e}
BHT	0.12±0.05	0.14±0.14	0.16±0.05	1.82±0.05	0.20±0.05	0.23±0.03	0.28±0.017	0.42 ^{d}
Means	0.22 ^{f}	0.299 ^{e}	0.433 ^{d}	0.85 ^{b}	0.760^{c}	0.887^{b}	1.10^{a}	

Different letters in columns and rows indicate significant different values at $P < 0.05$.

Results in Table 7 reveal that the antimicrobial effect of lyophilized pomegranate wastes depends on its added concentration. BHT addition caused slight reduction in microbial count of fresh patties. The rate of the microbial count increase was low in products having 2000 and 1500 ppm than 1000 and 500 ppm of pomegranate wastes extract during storage. Total microbial count reach to 10^{6} cfu/g after 12,16,and 20 day of storage at 4 ^{o} C for control, 500 ppm, both 1000 ppm and BHT pattie samples respectively. According to Jay (1986) foods having an aerobic total count less than 10^{5} CFU/ g have an acceptable quality while those with 10^{6} or more CFU/ g are generally have detectible off flavour. Both gallic and ellagic acids in the lyophilized polyphenols of pomegranate wastes extract were able to inactive the growth of bacteria. Hayrapetyan et al., (2012) found that, both acids are able to inactivate *B. subtilis , E. coli, L. monocytogenes and S. aureus.*

It can be concluded from the above results that polyphenols in the wastes of the pomegranate juice extraction can serve as a good natural bi-functional agent , an antioxidant and antimicrobial.

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مستخلص مخلفات عصير الرمان(*Punica granatum* L) كمادة حافظة للأغذية

فاتن فاروق عبد السلام ، يحيى جمال الدين محرم و عصمت صابر الزلاقى
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تم في هذه الدراسةتجهيز مستخلص خام مجفد من عديدات الفينول من مخلفات عصر حبوب الرمان وندرفول. و تم إستخدام هبتركيزات٢٠٠و ٥٠٠ و ٧٥٠ جزءفى المليون مقارناً مع مضاد الأكسدة المخلقة كيماوياًBHT عند تركيز ٢٠٠ جزءفى المليون لحفظ زيت عبادالشمس المكررتحت ظروف مشجعةللأكسدة , وأدت إضافة المستخلص المجفد لعديدالفينولات بنسبة ٧٥٠ جزءفى المليون الى خفض قيم أرقام البيروكسيد , وحمض الثيوباربيتيوريك وحموضةالزيت وتكوين مركباتDienes&Trienesبنسبة اكبر مقارناًبالتركيزات الأخرى والBHT. و كذلك تم قياس النشاط المضاد للميكروبات بطريقة الأنتشارعلى الاجارل لتعرف على قدرة مستخلص مخلفات الرمان المجفد بتركيزات بتركيزات صفر , ٥٠٠, ١٠٠٠, ١٥٠٠, ٢٠٠٠ جزءفى المليون كمضاد طبيعى للأكسدة على تثبيط خمسةمن السلالات الميكروبية الممرضة و هى; *Bacillus subtilis DB 100 host; Escherichia coli BA 12296; Klebsiellapneumoniae ATCC12296; Salmonella senftenbergATCC 8400* و *Staphylococcus aureusNCTC 10788* أدى إستخدام تركيز ٢٠٠٠ جزء فى المليون إلى خفض العدالكلى للبكتيريا