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Vol. 13(37), pp. 3812-3820, 10 September, 2014
DOI: 10.5897/AJB2014.14040
Article Number: F1EA87747392
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Improving oxidative stability of ghee using natural oxidants from agri-industrial wastes

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Received 10 July, 2014; Accepted 22 August, 2014

Bioactive compounds found in peanut skin (PS), pomegranate peels (PP) and olive pomace (OP) cake were extracted using ethanol (80%), ethyl acetate and hexane. The ethanol extract showed slightly better antioxidant characteristics than ethyl acetate and hexane extracts. Extracts showed varying degrees of antioxidant potential in different test systems in a dose-dependent manner. In general, it was observed that extracts with higher antioxidant capacity were in parallel to their higher phenolic contents. Total phenolic compounds (as gallic acid equivalent, GAE) ranged between 0.89 to 16.6, 1.83 to 261 and 1.56 to 124 mg GAE/g extract for OP, PS and PP, respectively. Ethanol extracts of different by-products were added to ghee at concentrations of 200, 400 and 600 ppm, respectively. BHA was also added to ghee at a concentration of 200 ppm. All samples were incubated at 63°C/21days. Ethanol extracts of PS, OP and PP gave good antioxidant activity during accelerated oxidative incubation of ghee. It could be concluded that ethanol extracts under study, at a concentration of 200 ppm, can retard fat auto-oxidation.

Key words: Ghee, agri industrial by-products, natural antioxidants, stability indices.

INTRODUCTION

Antioxidants (natural and synthetic) play a significant role in retarding lipid oxidation reactions in food products. The deterimental effects of excessive lipid oxidation such as formation of off-flavors and undesirable oxidized chemical compounds (aldehydes, ketones and organic acids etc) are well known (Saad et al., 2007). Synthetic antioxidants [for example, tertiary butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA) and butylated hydroxytolune (BHT)] are widely used as food additives, but their application has been reassessed because of possible toxic or carcinogenic components formed during their degradation (Jo et al., 2006; Pitchaon et al., 2007). Consequently, the search for endogenous protective ingredients in foods has been intensified wherein their utilization requires only manipulation of food formulations. A number of natural antioxidants have been added during

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food processing and have elongate the shelf life and oxidative stability of stored products (Chenn et al., 2008; Ebrahimabadi et al., 2010; Jang et al., 2012; Xiaowei et al., 2011).

A huge amount of plant biomass wastes is produced yearly as by-products from the agro-food industries. These wastes are attractive sources of natural antioxidants. The high concentration of phenolic compounds present in peels, skins and seeds supports the utilization of these residues as a source of natural antioxidants. Phenolic compounds exhibit a wide range of physiological properties such as anti-allergenic, anti-arthergenic, anti-inflammatory, anti-microbial, antioxidant, anti-thermobotic, cardio protective and vasodilatory effects (Balasundram et al., 2006). Phenolics could be extracted by water or solvents and the extraction conditions need to be optimized with respect to solvent polarity and physical conditions (Nepote et al., 2005). In addition, research has indicated that natural phenolic compounds can be extracted from raw materials or waste products of food industry (Peschel et al., 2006).

Studies were conducted to investigate antioxidants properties of peanut, peanut kernels, peanut hulls and peanut-based products (Yu et al., 2005; Wang et al., 2007). Peanut skins were demonstrated to be rich in phenolics and other health promoting compounds (Yu et al., 2005; Wang et al., 2007; Monagas et al., 2009). The olive oil industry generates large quantities of a deleterious by-product known as olive pomace. Olive pomace has broad spectrum toxicity against some microorganisms, plants, insects, animals and human cells (Obied, 2007; Aldini et al., 2006). Nevertheless, olive pomace has been considered as a potential source of natural antioxidants (Niaounakis and Halvadakis, 2004; Aldini et al., 2006). A wide range of phenolic compounds has been identified in virgin oil (Suarez et al., 2010) wherein only *ca*. 2% of the total phenols found in olive fruits are transferred to the extracted olive oil. The rest of phenolic compounds (98%) are retained in the olive waste cake. Pomegranate has been used extensively in the folk medicine of many cultures and its consumption has grown tremendously especially in the last decades (Li et al., 2006; Cam et al., 2009). The peels of some fruits have higher antioxidant activity than pulps (Guo et al., 2003; Fuhrman et al., 2005). Pomegranate is a good example for this type of fruits wherein their peels constitute approximately 40% of the whole fruit and are rich in ellagic acid derivatives (Cerda et al., 2003; Seeram et al., 2005).

From an environmental and economic perspective, it is very important that plant by-products produced by agro-food industry be used. Therefore, the objectives of this study were; (1) to evaluate different extracts from peanut skin (PS), pomegranate peels (PP) and olive waste cake (OP) as a source of natural antioxidants, (2) to characterize the composition and content of phenolics in different extracts and (3) to evaluate the efficiency of using agro food wastes ethanolic extracts in improving the quality, overall acceptance and oxidative stability of ghee during storage under thermal oxidative conditions.

MATERIALS AND METHODS

Plant biomass wastes, as a by-products of food industries, commonly found in Egypt, were used in this investigation. Olive (*Olea europaeal* L.) pomace (OP) was obtained from Food Technology Research Institute (Agricultural Research Center, Giza, Egypt). Peanut (*Arachis hypogaea* L.) skins (PS) were obtained from the 10th of Ramadan City. Pomegranate (*Punica granatum* L.) fruits (PP), were obtained from local market (Zagazig, Egypt), washed with distilled water and manually peeled. Pomegranate peels (PP) were collected then rinsed with distilled water and considered as a by-product. The starting materials were dried in an air draft drying oven (40°C) until the moisture content became 12% or less. By-products were ground and sieved through 60 mesh sieve and finally cooled or kept at 4°C until the extractions were carried out. Butylated hydroxyl anisole (BHA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid and quercin were purchased from Sigma (St. Louis, MO, USA). All other chemicals and reagents were of the highest purity available.

Preparation of extracts

Dried materials were extracted with different solvents, named ethanol (80%), ethyl acetate and hexane at a ratio of 10:1 (v/w, 10 mL solvent: 1 g raw material) in closed vessels by stirring at room temperature (25°C) for 4 h followed by filtration through Whatmann no. 1 filter paper. The residues were re-extracted again under the same conditions.

All vessels were wrapped with aluminum foil to prevent light degradation during extraction (Yu et al., 2005). N-Hexane and ethyl acetate extracts were evaporated in a rotary evaporator (Buchi-water bath-B-480, Switzerland) below 40°C, while ethanol 80% extracts were freeze-dried (Thermo Electron Corporation-Heto Power Dry LL 300 Freeze Dryer, Gzechoslovak). The dried extracts after evaporation of solvents were weighed to determine the yield and stored at -20°C until used.

Determination of total phenolic compounds (TPC)

The concentration of TPC in different extracts was measured using UV spectrophotometer (Jenway-UV-VIS Spectrophotometer), based on a colorimetric oxidation/reduction reaction, as described by Škerget et al. (2005) using Folin-Ciocalteu reagent. Specifically, 0.5 ml of diluted extract (10 mg in 10 ml solvent) was mixed with 2.5ml of Folin-Ciocalteu reagent (diluted 10 times with distilled water) and 2 ml of Na_2CO_3 (75 mg/ml). The sample was incubated for 5 min at 50°C then cooled. For a control sample, 0.5 ml of distilled water was used. The absorbance was measured at 760 nm. Total phenolic content expressed as gallic acid equivalent (GAE) was calculated, and the results were expressed as a mg GAE /g extract.

Identification of phenolic acids using HPLC

Phenolic acids of the dried extracts were identified according to the method described by Mattila et al. (2000). HPLC (Hewllet Packard series 1050, USA) equipped with auto sampling, injector, solvent degasser, UV detector set at 330 nm and quarter HP pump (series 1050) was used. Column (C_{18} hypersil BDS) with particle size 5 μm was used. The separation was carried out with methanol and

Table 1. Yield of extracts (g/100 g) for different plant wastes-by products of food processing.

Plant waste	Hexane extract	Ethylacetate extract	Ethanol 80% extract
Olive pomace	5.23	1.00	9.43
Pomegranate peels	0.50	0.90	43.84
Peanut skin	9.47	7.67	14.56

Table 2. Total phenolic compounds (mg gallic acid /g extract) in different extracts.

By-product	Concentration (mg gallic acid/ g extract)		
	Hexane	Ethyl acetate	Ethanol 80%
Olive pomace (OP)	16.63	0.89	12.23
Pomegranate peels (PP)	1.56	12.49	124.23
Peanut skins (PS)	1.83	5.69	261.69

acetonitrile as a mobile phase at a flow rate of 1mL/min. The column temperature was performed at room temperature (25°C) throughout the experiment. Identification and quantification were carried out based on calibrations of the standards prepared from phenolic acids dissolved in a mobile phase. Retention time and peak area were used for calculation of phenolic acid compounds by the data analysis of Hewllet Packared Software.

Radical scavenging activity (RSA) of extracts

The electron donation ability of the obtained extracts was measured by bleaching of the purple colored solution of DPPH according to the method of Hanato et al. (1988). 100 µL of each extracts (10 mg extract/10 ml solvent) was added to 3 ml of 0.1 mM DPPH dissolved in ethyl acetate, ethanol and hexane according to the solvent used for extraction. After an incubation period of 30, 60 and 120 min at room temperature, the absorbance was determined against a control at 517nm (Gulcin et al., 2004). Percentage of antioxidant activity of DPPH was calculated as follows:

$$\text{Antioxidant activity (Inhibition) \%} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where, $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of the extract. BHA was used as a positive control. Samples were analyzed in triplicate.

Stability of ghee enriched with ethanol 80% extract

The butter, used for preparing ghee in the present study, was made from pasteurized and un-ripened buffaloes' cream. The butter was converted into ghee by boiling according to the method described by Fahmi (1961). Ghee samples were divided into 11 portions as follows: Portion 1 was kept without additives as control throughout the experimental period and was considered to be as a negative control (C); Portion 2 was treated with 200 ppm BHA throughout the experimental period of three weeks and was considered to be as positive control (C_1); portions 3, 4 and 5 were treated with 200, 400 and 600 ppm of PS ethanolic extract, respectively, (T_1, T_2 and T_3).

Portions 6, 7 and 8 were treated with 200, 400 and 600 ppm of PP ethanolic extract, respectively, (T_4, T_5 and T_6). Portions (9, 10 and 11) were treated with 200, 400 and 600 ppm of OP ethanolic extract, respectively, (T_7, T_8 and T_9).

All samples were incubated in an oven at 63±1°C to accelerate the oxidation for 21 days. Samples were analyzed every three days for peroxide value (PV), acid value (AV), and 2-thiobarbituric acid (TBA) value. AV and PV were determined according to AOAC (1984). Determination of lipid oxidation was assessed in triplicates by the TBA method according to Fernandez-Lopez et al. (2005).

Oxidation stability test of Ghee

Determination of an oxidative stability of ghee by Rancimat equipment (USA, model 617), was based on volatile acids from oxidation reaction passed through DI water, in which conductivity values were detected. Heating block was held constant at 130°C. A rate of air flow through liquid butter oil (ghee) was 10 L/h. Prior to the testing, frozen samples of ghee were thawed at 40-50°C, and a 3±0.002 g of ghee sample was taken for the analysis according to AOCS (1997).

RESULTS AND DISCUSSION

Characterization of phenolic compounds TPC in different extracts

The yield of extracts with different solvents varied from 0.1 to 42.5 g extract/100 g wastes (Table 1). PS, PP and OP had the highest yield when extracted with ethanol 80% followed by hexane and ethyl acetate, respectively. Variation in the extraction yields of different extracts might be attributed to differences in polarity of compounds found in plants such differences have been reported (Jaya et al., 2001).

The amount of TPC varied in the different extracts, ranging from 0.89 to 261 mg GAE/g extract (Table 2). In general, the results stated that ethanol 80% and ethyl acetate were better than hexane in extracting phenolics from PP and PS owing to their higher polarity and good solubility (Siddhuraju and Becker, 2003; Kequan and Liangli, 2004). On the other side, hexane extracted the highest amount of phenolics from OP followed by ethanol 80% as shown in Table 2.

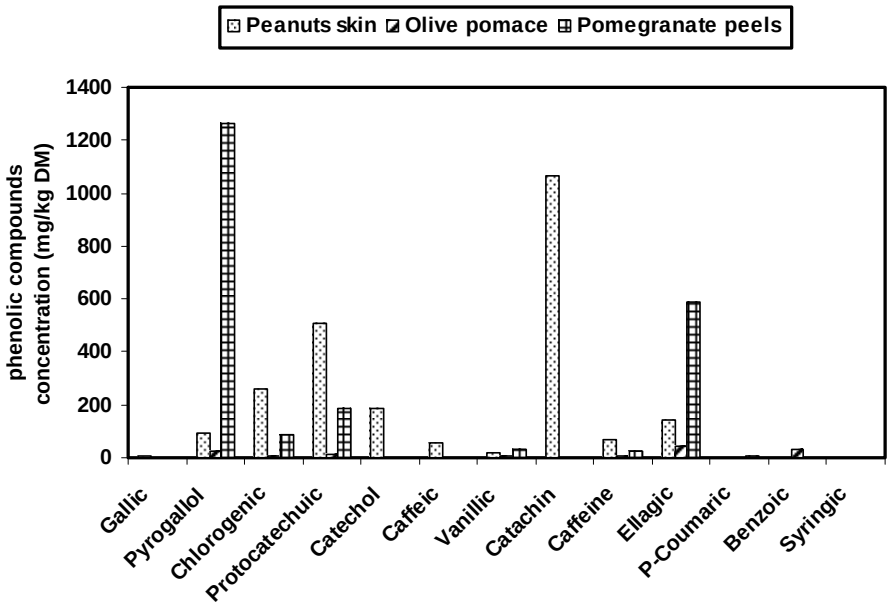

Figure 1. Levels of phenolic compounds in plant wastes-by products of processing food extract as determined by HPLC.

Figure 1 shows the percentage of identified phenolic compounds in PS, PP and OP. There was a great variation among the components identified in each waste by-product. Phenolic compounds were identified in PS, namely pyrogallol, protocatechuic, catachin and ellagic acid, with amounts ranging from 0.07 to 10.64 mg/g. The main phenolics identified in OP were pyrogallol, ellagic acid, chlorogenic, protocatechuic with amount ranging from 0.01 to 0.46mg/g. The major phenolic compounds identified in PP were pyrogallol, ellagic acid, chlorogenic, protocatechuic with amount ranging from 0.05 to 12.64 mg/g. Balasundran et al. (2006) stated that the antioxidant activity of phenolic compounds depends on the structure, in particular the number and positions of the hydroxyl groups of the nature of substitution on the aromatic rings. Moure et al. (2001) reported that the antioxidant compounds from residual sources could be used for increasing the stability of foods by preventing lipid peroxidation and also for protecting oxidative damage in living systems by scavenging oxygen radicals. It is well known that total antioxidant activity of waste extracts was line early proportional to the concentration of total phenolics (Meftahizade et al., 2011).

RSA against DPPH•

The results of RSA of various extracts are represented in Figure 2. The results clearly indicate that all extracts exhibited antioxidant activity. The extracts that contained a high amount of TPC (Table 2) showed high RSA. In general, ethanol 80% followed by hexane then ethyl acetate extracts showed RSA as strong as that of BHA (Figure 3A, B and C). It has been proven that the antioxidant activity of plant extracts is mainly ascribable to the concentration of phenolic compounds in the plant (Heim et al., 2002). The extracts RSA with different solvents varied from 91.4 to 5.50% after 120 min of incubation.

The highest RSA was observed with PP ethanol 80%, hexane and ethyl acetate extracts with respective values of 80, 38 and 35%, respectively. Ethanol 80%, hexane and ethyl acetate extracts of PS had values of 85, 22 and 19%, respectively. In addition, hexane, ethanol 80% and ethyl acetate extracts of OP had values of 24, 20 and 18%, respectively.

The results of the DPPH$^{\bullet}$ radical scavenging assay suggest that components involving the extracts are capable of scavenging free radicals *via* electron- or hydrogen-donating mechanisms and thus might be able to prevent the initiation of deleterious free radical mediated chain reactions in susceptible matrices. This further shows the capability of the extracts to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathological damage. The effect of antioxidants on DPPH radical-scavenging is thought to be due to their hydrogen-donating ability, DPPH$^{\bullet}$ is a stable free radical and accepts an electron or hydrogen radical to become a stable molecule (Gulcin et al., 2004). Free radicals involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies such as cancer and cardiovascular diseases (Dorman et al., 2003).

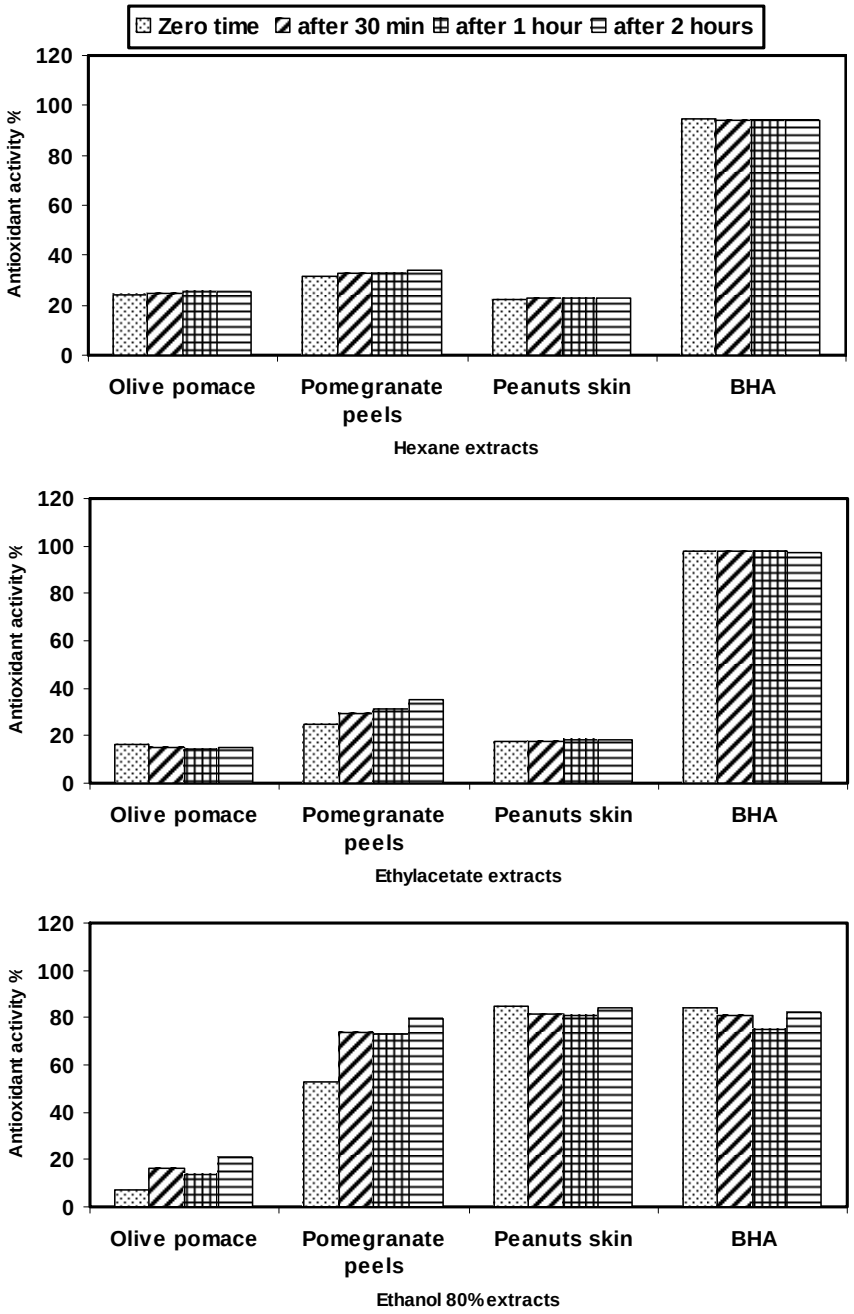

Figure 2. RSA of hexane (a), ethyl acetate (b) and ethanol 80% (c) wastes extracts in DPPH• radical scavenging activity system compared with BHA.

Stability of enriched-ghee during storage under accelerated oxidative conditions

Peroxide values

Data illustrated in Figure 3 shows that the PV of control ghee samples increased during the accelerated incubation up to 21 days. The others samples enriched with BHA and natural antioxidants had the lower PV values than the control sample during storage under accelerated incubation at 63°C/21days. The results obtained in this work reflected the impact of these extracts, as natural antioxidants, in the retarding of ghee oxidation. These results are in agreement with those of Puravankara et al. (2000) and Pankaj et al. (2013). The order of efficiency in inhibiting oxidation was in the order PS, PP and finally OP extract.

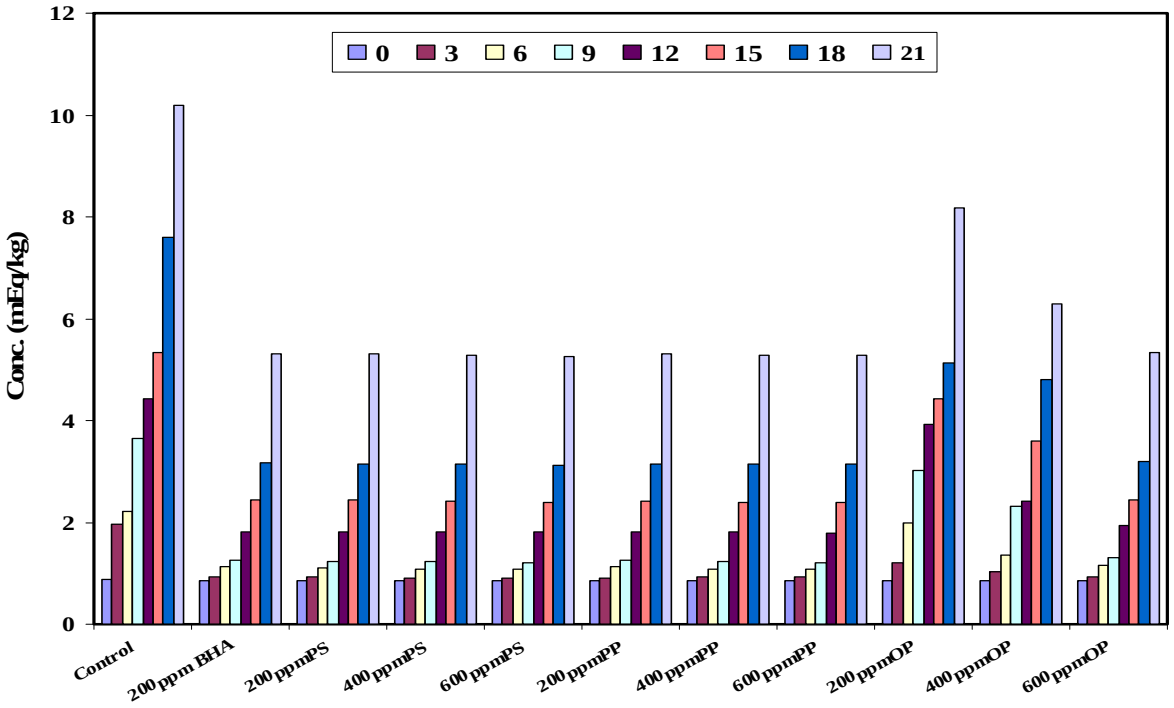

Figure 3. Effect of ethanol extracts at different concentration on the peroxide value of Ghee during storage.

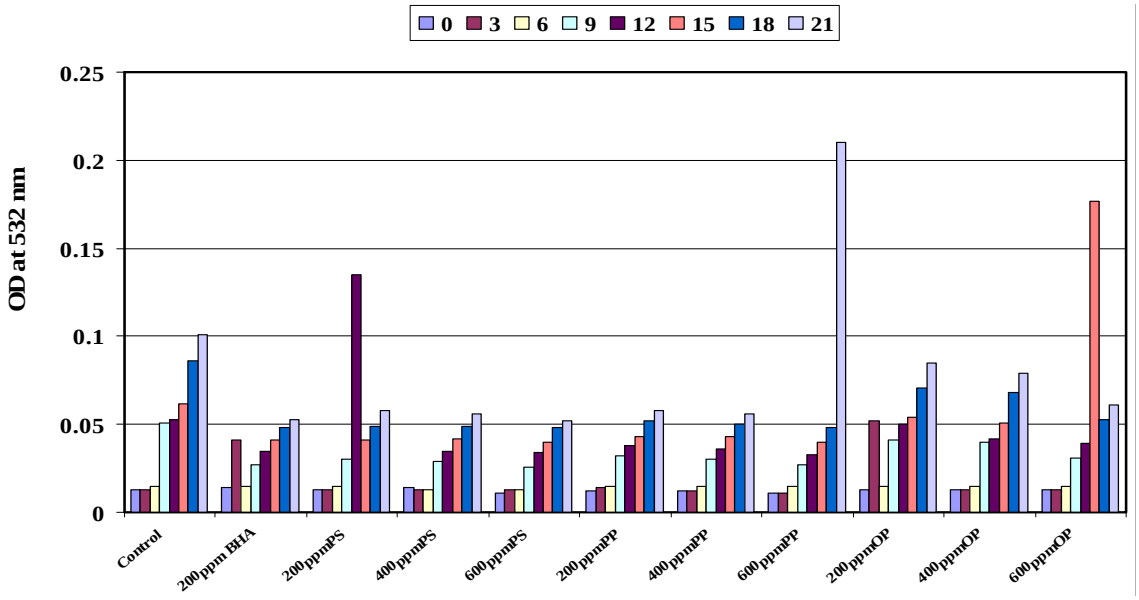

Figure 4. Effect of ethanol extracts at different concentration on the TBA of Ghee during storage.

TBA

It is well known that TBA values are taken as an index to evaluate the advance of oxidation changes occurred in oil and fats. The addition of extracts as natural antioxidants to ghee retarded the oxidative changes during accelerated storage (Figure 4). This means that the formation of malonaldehyde, which affect the formation of pink colour intensity from the reaction of TBA material with malonaldehyde, took place at a relatively lower rate in treated ghee samples. However, the control ghee samples showed higher TBA values throughout the

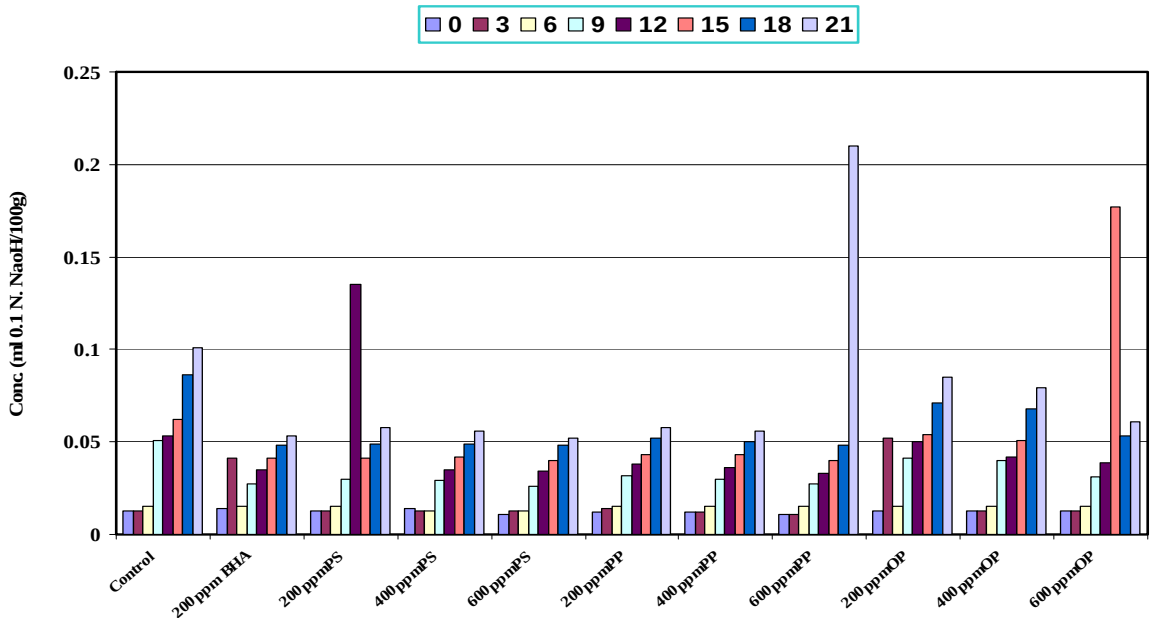

Figure 5. Effect of ethanolic extracts at different concentrations on acid value of Ghee during storage.

accelerated incubation period. These structural requirements were supported by the powerful antioxidant activity of the well-known BHA. Phenolic compounds act as hydrogen or electron donors to the reaction mixture and therefore the formation of hydro peroxides are decreased. The slow formation of conjugated dienes and consequently the secondary products by extracts and their major compounds indicated that these materials may act as hydrogen donors to proxy radicals, thus, retarding the autoxidation of linoleic acid by chain radical termination (Farag et al., 1989; Özkanlı and Kaya, 2007; Mohdali, 2010).

Acid value

Data illustrated in Figure 5 shows that the AV remained without noticeable changes within the first six days of storage at 63°C for all treatments including the control. Slightly increases in AV were observed until 15 days of storage period, then considerable increase in AV were recorded till the end of storage period (21 days) for all samples including the control. Data presented in Figure 3 show clearly that the AV of stored ghee was noticeably affected by enrichment with by-products ethanolic extracts. PS ethanol 80% extracts (200 ppm) showed the lowest increase in AV compared with other extracts and BHA at same ratio (200 ppm). These results are in agreement with those of Siddhuraju and Becker (2003).

Rancimat

The results of induction period are illustrated in Figure 6. Induction time was the highest (24.3 h) for ghee enriched with BHA, followed by ghee enriched with PS (18.4 h) and was the lowest for control (15.3 h). These results agree with those of Marian Kucera et al. (2011) and Suwarat and Tungjaroenchai (2013). As can be seen from Figure 6, the logarithm of TRanc shows linear dependence on exothermic temperature and can be described by the following equation: (Log TRanc = a . t + b), where: a and b are adjustable coefficients and T the temperature in degree celsuis (°C). The oil stability index OSI of ghee samples varied from 14.85±0.17 to 24.80±0.34 h; the higher the temperature and longer period of time, the higher were values of OSI in hours (Figure 6). Although some papers reported that high temperat ure with prolong period of time affected high yield, color, oxidative stability tended to changed continuously. Degradation of oil was normally induced by moisture, high temperature, crust formation and various structural, textural and chemical changes in the product, and degradation of frying medium (Paul et al., 1997). The oxidative stability of oils can be ranked based on the rule "the longer the induction time the more stable is the oil". The relative resistances of the oils on their thermal oxidative degradation depend on temperature.

Conclusion

Peanut skin, pomegranate peels and olive pomace extracts were prepared using different solvents, and the *in vitro* antioxidant activity of each extract was investigated. In general, it was observed that extracts with higher antioxidant capacity were in parallel to their

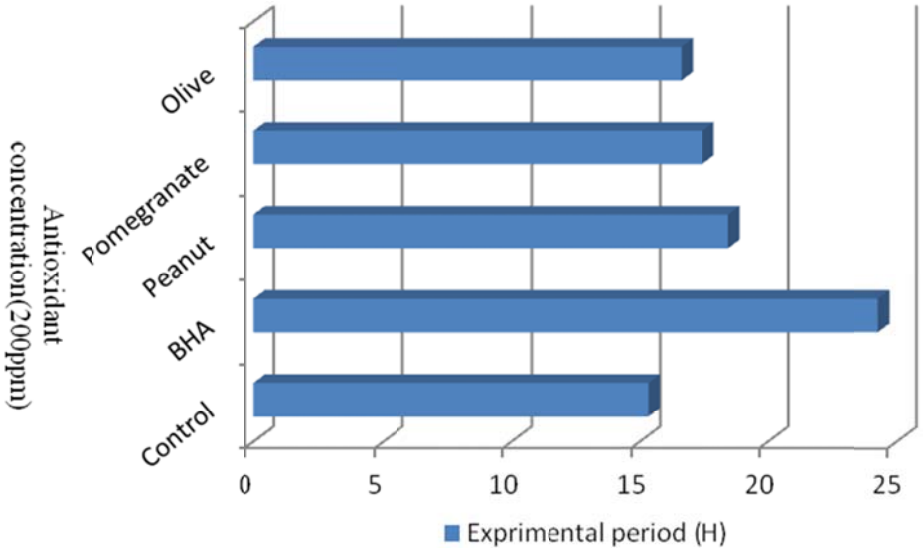

Figure 6. Rancimat of ghee treated with different plant wastes-by products of food processing extracts at 130°C.

higher phenolic contents. It could be concluded that the obtained extracts using higher-polarity solvents were more effective radical scavengers than those obtained using lower-polarity solvents. Ethanol 80% showed slightly better characteristics than hexane and ethyl acetate as a solvent for phenolic compounds extraction. Thus, for use in the food industry, ethanol 80% would be a more appropriate solvent. Furthermore, it is notable that PS extracts exhibited a strong antioxidant capacity in all assays used, followed by PP and OP extracts. Overall, ethanol 80%, ethyl acetate and hexane extract showed relatively comparable activity to BHA. Therefore, these extracts could be used as preservative ingredients in the food and/or pharmaceutical industries.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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