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Utilization of grape pomace extract as a source of natural antioxidant in biscuits

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Abstract

In the present study, grape pomace extract (GPE) was employed as a source of natural antioxidants to reduce lipid oxidation in the food matrix. The total phenolic of GPE was investigated by three various solvents (methanol, ethanol, and acetone). Individual phenolic compounds by HPLC and antioxidant capability, including 1,1-diphenyl-2-picryhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) tests, were also evaluated. The obtained results showed that GPE owned good antioxidant activity, which gives DPPH of 86.89% and FRAP of 73.93%. Peroxide values (PV), free fatty acids (FFA), and thiobarbituric acid (TBA) were applied as measures to assess the antioxidant efficacy of GPE (0.5%, 1%, 2% and 3%) in biscuits. At the end of 6 months storage period of biscuits, it was observed that increasing the GPE concentration enhanced the capability of inhibiting lipid oxidation. The findings from this work

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demonstrate that GPE at various levels exhibited very powerful antioxidant ability. Therefore, grape pomace extract in fatty food products could be utilized as a natural antioxidant to reduce lipid oxidation, which can be useful for future food applications.

Key words: Grape pomace extract; antioxidant activity; phenolic acids; biscuits; lipid oxidation.

1. INTRODUCTION

Grape pomace (GP) is a significant by-product recovered throughout the processing of grape products (wine, juice) and consists of seed, skin, and stems. Grape pomace represents one of the major environmental wastes globally (González-Centeno et al., 2010). However, this pomace contains a substantial quantity of bioactive polyphenolic components, which could be utilized as a great potential functional food or dietary supplements for human health (Kammerer, Claus, Carle, & Schieber, 2004). To recover phenolic components from GP waste, various methods have been reported by other researchers. Such methods are as follows; solvent extraction (Amendola, De Faveri, & Spigno, 2010), ultrasounds (González-Centeno, Comas-Serra, Femenia, Rosselló, & Simal, 2015), microwave heating (Pedroza, Amendola, Maggi, Zalacain, De Faveri, & Spigno, 2015) and enzyme digestion (Binaschi, Garrido, Cirelli, & Spigno, 2018). The isolated extracts can be employed in the production of novel food products as natural antioxidant additives (Binaschi et al., 2018).

Major significant derivation from cereal-based products for functionalization is well studied, as well as with components from grape-making by-products (Lavelli, Torri, Zeppa, Fiori, & Spigno, 2016). Amongst cereal-based products, biscuits are one of the most commonly consumed bakery finished products in the world. Such extensive demand is culminating from the ubiquitous snack, readily available for consumption, affordability, high nutritional quality, high flavor density, and stable shelf life (Rowayshed, Sharaf, El-faham, Ashour, & Zaky, 2015). Recently, food manufacturing and the bakery food sector are seeking novel recipes to produce healthier foods that

meet consumers' requirements (Pinto, Castro, Vicente, Bourbon, & Cerqueira, 2014).

There are a few reports about the enhancement of biscuits with grape wastes or relevant extracts (Davidov-Pardo et al., 2012; Maner, Sharma, & Banerjee, 2017). Therefore, the current study was undertaken to examine the utilization of GP as a source of natural antioxidants and its efficiency in the inhibition of fat rancidity in biscuits. Total and individual phenolic contents, as well as antioxidant activity in grape pomace extracts, were also evaluated.

2. MATERIALS AND METHODS

2.1. Materials

Grape pomace was supported by Ganaklis Factory for Beverages (Alexandria, Egypt). Soft wheat flour, sugar powder, skimmed milk powder, bakery fat, and eggs were purchased from a local supermarket (Giza, Egypt). Food grade dextrose, salt, ammonium bicarbonate, and sodium bicarbonate were utilized in biscuit processing. 1,1-diphenyl-2-picryhydrazyl (DPPH), Folin-Ciocalteu Reagent, TPTZ (2,4,6-Tris (2- pyridyl)-s-triazine) and phenols standard (chlorogenic acid, caffeic acid, gallic acid, syringic acid, sinapic acid, isoferulic acid, ferulic acid, protocatechuic acid, isovanillic acid, vanillic acid, catechin, tyrosol, apigenin, and quercetin were gained from the Sigma (St. Louis, MO, USA). The other chemicals employed were of analytical grade.

2.2. Analytical Methods

2.2.1 Proximate composition

Moisture, protein, fat, ash, and fiber content of grape pomace waste were conducted according to the techniques outlined by (AOAC, 2016).

2.2.2 Preparation of grape pomace extract (GPE)

Extracts of the sample were accomplished according to the method of El-Faham, Mohsen, Ashour, Sharaf, & Zaky (2016). Grape pomace (GP) was dried in an air oven at 50ºC. The dried sample was ground into a soft powder and sieved. For extraction, 2 g of powder sample was dispersed in 20 mL of organic solvents (methanol, ethanol, and acetone) overnight in a shaker at ambient temperature. Afterward,

the extracts were filtered through filter paper (Whatman No.1), and the sediments were re-extracted through the same procedure. The mixtures (40 mL) were collected and concentrated to remove organic solvent at 40°C using a rotary evaporator (Buchi Rotavapor, Switzerland). The recovered extracts were stored at -20°C for analysis.

2.2.3 Total phenolic content (TPC)

The TPC of extracts was carried out according to the technique outlined by Rowayshed et al. (2015). A 20 µL of the sample was diluted with 1.58 mL of MilliQ water, and then Folin-Ciocalteu reagent (100 µL) was mixed. After 3 min. 300 µL of 20% sodium carbonate was mixed. The solution was incubated for 30 min in the dark. After, the absorbance was measured at 765 nm (Cary 60 spectrophotometer, Agilent Technologies, USA). Gallic acid was used as a reference to construct a standard curve. The data were displayed as mg GAE per 100g sample.

2.2.4 Antioxidant activity

2.2.4.1 DPPH-radical scavenging activity

The DPPH ability assay was investigated using the technique described by El-Faham et al. (2016). Forty microliters of GPE were merged with 2900 µL DPPH (0.1 mM in 80% methanol). The solution was put in the dark for 30 min. To estimate the DPPH activity, the absorbance of the findings was controlled at 517 nm, and the following Eq. was employed:

$$\text{DPPH activity (\%)} = 1 - (\text{Abs}_{\text{sample}}/\text{Abs}_{\text{control}}) \times 100.$$

2.2.4.2 Ferric reducing antioxidant power (FRAP)

The FRAP test was performed according to the report of Benzie and Strain, (1996). The working solution was prepared by combining 0.1 mol L^{-1} acetate buffer (pH 3.6), 10 mmol L^{-1} tripyridyltriazine (TPTZ) in 40 mmol L^{-1} hydrochloric acid, and 20 mmol L^{-1} ferric chloride (10:1:1, v/v/v). To assess its ferric reducing power, the absorbance of the reaction solution, which was consist of a 3 mL working solution and a 100 µL sample solution was controlled at 593 nm after standing for 10 min at ambient temperature. A standard curve was provided using freshly prepared ammonium ferrous sulphate (25–1600 µM Fe^{+3}).

2.2.5 Determination of phenolic composition

The individual phenolic compounds of GPE were evaluated using an Agilent HPLC 1260 Infinity II system (Agilent Technologies, USA). An Agilent Zorbox SB-C18 column (250 × 4.6 mm i.d., 5 µm) was used at a column temperature of 30ºC. The filtered sample (20 µL) was injected and the mobile phase was consisted of 0.1% formic acid (A) and methanol (B) with a flow rate of 1.0 mL/min. The gradient was set as follows: 0 minutes 25% B; 20 minutes 25% B; 30 minutes 35% B; 40 minutes 100% B; 42 minutes 100% B; 50 minutes 25% B. The peaks of chromatogram were detected at 280 nm. The levels of each compound were quantified based on a standard curve, and the values were displayed as mg per 100 g DW.

2.3 Technological application

2.3.1 Preparation and processing of biscuits

The biscuit recipe employed was as follows: wheat flour (100 g), 30 g powdered sucrose, 15 g butter-milk, 24 g eggs, 0.93 g salt, 1.11 g of sodium bicarbonate, 3 g ammonium bicarbonate, 18 g skimmed milk powder, 3 g baking powder, 0.5 g vanilla and the required volume of water. Because methanol and acetone are not safe to be used in food applications, the ethanolic GPE was selected for the further step after removing its residues. The substitution of wheat flour with GPE was conducted based on (0, .5%, 1%, 2%, and 3%) of the wheat flour weight. Wheat flour without any additives as control and BHA (200 ppm) samples were also prepared. After that, biscuits were processed according to the process outlined by Rowayshed et al. (2015) and baked for 8-10 min at 205° C. After baking; various biscuit samples were cooled and collected in low-density polyethylene (LDPE) bags, sealed and kept at room temperature for 6 months until further investigation.

2.3.2 Physical attributes of biscuits

Diameter (W), thickness (T), and the spread ratio of treatments were done using standard techniques AACC (2000) 10-50D.

2.3.3 Color evaluation

The color analysis was carried out on the surface of biscuits using (Hunter, Lab Scan XE- Reston VA, USA).

2.3.4 Sensory evaluation

Sensory attributes of biscuits were assessed according to the procedure reported by Reddy et al. (2005) with minor modification. The sensory evaluation of biscuits (freshly prepared and stocked) was carried out to estimate the degree of acceptability of the products prepared by the addition of GPE. Twenty panelists (10 males and 10 females) were chosen from the members of the Food Technology Department, National Research Centre, Egypt. Sensory scores for various properties such as color, texture, taste, odor, and overall quality were received using a 9-point hedonic scale with anchors from 1 = dislike extremely to 9 = like extremely. Panelists attended 1-h training sessions that were accomplished by scientists with commercial samples for one week. Samples were individually presented in paper plates, and recognized with three-digit code numbers. Samples were served at room temperature in random order. Water was also given to panelists to rinse their mouths between samples.

2.3.5 Measurement of peroxide value (PV)

Peroxide value of treatments was assessed according to AOCS official method Cd 3d-63 (1996).

2.3.6 Free Fatty Acids (FFA)

Titrimetry was classically employed to define the acid value (free fatty acid content) of fats. The acid value was evaluated according to the method described in AOCS Official Method Cd 8- 53 (1996).

2.3.7 Thiobarbituric Acid Test (TBA)

Malonaldehyde formation as an index of secondary lipid peroxidation was conducted using the procedure outlined by Norhidayah, Babji, Shazali, Norazmir, and Norazlanshah (2011).

2.8 Statistical analysis

All tests were accomplished in triplicate. Results were presented as means ± SD. The findings were achieved by applying SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Statistical differences amongst samples were identified utilizing Duncan's multiple-range test ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Proximate composition

The whole composition of grape pomace waste are exhibited in Table 1, where grape pomace recorded 62.79 ± 0.13% moisture, 6.07 ± 0.03% fat, 12.26 ± 0.45% of crude protein, 8.49± 0.01% ash, 11.58 ± 0.34% crude fiber, and 61.60% carbohydrates by difference. These findings were in line with those reported by Beres et al. (2016) and which possibly support the fact that grape pomace extract might be utilized as a source of protein, fiber, and carbohydrate.

3.2. Extraction yield

Solvent extraction is more commonly employed for the isolation of polyphenols. Both extraction yield and antioxidant capacity of extracts significantly depend on the solvent type. The extraction yield of grape pomace extracts by three different solvents was shown in Table 2. The methanolic extract exhibited the highest yield (7.22 ± 0.08), followed by ethanolic and acetone (4.16 ± 0.21 and 2.46 ± 0.53%, respectively) ($p < 0.05$). Such differences in the various extracts yields are associated with discrepancies in compounds polarity existing in plants, and these differences have been stated in work on fruit seeds (Jayaprakasha, Singh, & Sakariah, 2001). A significant difference ($p < 0.05$) in extraction yields for the tested sample with various solvents was observed.

3.3. Total phenolic content (TPC)

The TPC of grape pomace waste and its various solvent extracts are given in Table 2. The data revealed that methanol was the great solvent for phenolic components extraction, followed by ethanol and acetone, with values of 327.71 ± 0.67, 205.53 ± 0.11, and 104.2 ± 0.33 mg GAE $100g^{-1}$ DW, respectively ($p < 0.05$). This variation can be related to a higher polarity and best solubility of phenolics with methanol, which enhanced the phenolic content (El-Faham et al., 2016). The polyphenols content in grape wastes might differ due to several factors such as different cultivars, climate, harvest time, and the environment of growth (Antoniolli, Fontana, Piccoli, & Bottini, 2015).

3.4. Antioxidant activity

3.4.1. DPPH-radical scavenging activity

The grape pomace extract obtained by methanolic treatment owned the capacity to scavenge DPPH radicals (Table 3). The results displayed that the grape pomace possessed higher DPPH (67.65%) than BHA (65.20%). The growth in the free radical scavenging might be owing to a rise in the polyphenols contents, as presented in Table 2. This is mainly due to its high hydrogen donating capacity. This notion is in harmony with Ajila, Aalami, Leelavathi, and Rao (2010). Also, our findings were higher than those found by Negro, Tommasi, and Miceli (2003), but lower than those stated for other grape varieties such as Manto Negro red grape pomace (Llobera & Canellas, 2007). Our results revealed that the values of the DPPH ability of the tested samples were significant differences ($p < 0.05$).

3.4.2. FRAP assay

The FRAP test is usually employed to examine the antioxidant ability of plant stuff. The antioxidant capability of plant extracts is examined by the ability of the antioxidants to reduce ferric to ferrous iron form utilizing the FRAP system (Benzie & Strain, 1996). The FRAP of grape pomace extract and BHA control are presented in Table 3. Equivalent to DPPH activity values, grape pomace extract exhibited greater FRAP efficacy (73.93 mmol Fe_2SO_4 /100g) than BHA (52.46 mmol Fe_2SO_4 /100g) ($p < 0.05$). Our results revealed that the reducing power of pomace was greater than of red grape extract (27.39 mmol TEAC/100g), which found by Pérez-Jiménez et al. (2008).

3.5. Individual phenolic compounds by HPLC

HPLC is the most frequently applied for separation and identification of individual phenolic compounds due to its high-separation capability and relative simplicity. The phenolic acid profile of grape pomace extract is presented in Table 4. Protocatechuic acid was the highest polyphenol in pomace extract followed by gallic acid, while caffeic acid was the lowest amount obtained in pomace extract. These data are in agreement with Dumitriu, Peinado, Peinado, and López de Lerma (2015), who mentioned that protocatechuic acid and gallic acid were found to be the dominant phenolic acids in grape pomace. On the other hand, a moderate amount of chlorogenic acid, vanillic acid, and

quercetin (6.44, 6.0 and 4.07 mg/100g DW) were found in this study, respectively. These results confirmed that grape pomace is a good source of phenolics. Previous studies reported that phenolic compounds such as hydroxybenzoic, chlorogenic, sinapic and p-coumaric acids containing significant antioxidant activities (Zhang, Liao, Moore, Wu, & Wang, 2009).

Among the flavonoids detected, the results revealed that catechin was the predominant flavonoids in the extract followed by quercetin. The presence of catechin and quercetin in grape pomace being free flavonoids were informed by various reports (Montealegre, Peces, Vozmediano, Gascueña, & Romero, 2006; Rockenbach et al., 2011). Generally, variations in phenolic acid composition might be due to extraction chemicals and procedures utilized by various authors (Oroian and Sorina, 2017; Zaky, Chen, Liu, Li, & Jia, 2019). Finally, the HPLC results, as mentioned above, indicate that such phenolic rich extract from grape pomace may inhibit the oxidation of vegetable oils and fats.

3.6. Application of GPE in Biscuits

3.6.1. Physical measurements of biscuits

The effect of grape pomace extract on physical attributes of biscuits processed using 0.5, 1, 2, and 3% of GPE was studied, and the data are given in Table 5. The adding of GPE increased the diameter of the biscuits from 6.76 to 7.09 cm. The highest diameter (7.09 cm) was found by GPE at 3%, with no significant difference observed among 0.5, 1, and 2% GPE. It was also noticeable that using GPE at all levels in biscuit elaboration led to a decrease in thickness in comparison with control treatment (0.89 cm). The variations in diameter and thickness of treatments with the addition of GPE might be ascribed to the dilution of gluten. This concept is in accordance with those supported by Ajila, Leelavathi, and Rao (2008).

Regarding the spread ratio, it was remarked that substituting 3% wheat flour by GPE exhibited the highest value (10.12) when compared with other treatments.

3.6.2. Color of biscuits

Food color is one of the principal factors for buyers' acceptability. The influence of the adding of GPE on biscuit color was assessed (Table 6).

The L^* and a^* values diminished with the development in the levels of GPE compared to control. The difference in b^* value, which means the yellowness, progressively reduced with the rise in GPE concentration. The reduction in brightness and yellowness may be attributed to the enzymatic browning occurring through the biscuits processing (Ajila et al., 2008).

3.6.3. Sensory Evaluation of Biscuits

The biscuits enriched with GPE were subjected to the organoleptic test. The addition of GPE was observed lower in all feature parameters than control (Table 7). It was noted that the surface color of biscuits incorporating up to 1% of GPE was as agreeable as those of control biscuits. Results stated that incorporation of 3% GPE in biscuits produced a comparatively dark color, the development in darkness was displayed on L^* scores (Table 6), which could be related to the enzymatic browning. Aziah and Gomathi (2009) revealed that mango peels promoted a deep brown color to cookies; this might have given the judges an impression of an over-baked product, hence influencing their desires. Data also revealed that biscuits possessed pleasant texture with increased levels of GPE up to 1% when compared to control and BHA.

Furthermore, textural characterization plays a meaningful role in proving the overall acceptability of cookies. The taste and odor of biscuits were varied with the adding of GPE. However, at levels of 2% and 3% of GPE, the biscuits possessed a slightly sour taste, which might be owing to high polyphenols content. These data are in consonance with Maner, Sharma, and Banerjee (2017).

Among the overall quality, it could be observed that biscuits enriched with GPE up to 1% gave good records. The lower score values of GPE could be attributed to the barren color and the disagreeable taste. It could be assumed that biscuits with good overall quality can be provided by replaced 0.5% of wheat flour with GPE.

3.6.4. Peroxide value (PV)

The PV is one of the official extensively-employed experiments for the estimation of oxidative rancidity in fatty food (Shahidi & Wanasundara, 2002). A continuous growing in PV with the progress in the storage period was noted for all various treatment groups

(Figure 1a). The PV rate was extremely slow initially but recorded a significant rise after two months of storage period. Biscuits treatments except for antioxidant (control) owned the greatest peroxide value 21.84 meq kg^{-1} at the end of the storage period compared to other samples. PV was in the scope of 1.38±0.03-12.11±0.04 meq kg^{-1} for using GPE, whereas it was 1.45±0.03-15.13±0.05 meq kg^{-1} for biscuits BHA (200 ppm). A significant difference ($p < 0.05$) in PV was noted among the control and biscuits treatments, which reduced the peroxide rate. The PV of biscuits incorporating with 0.5, 1, 2, 3% of GPE and BHA were reached to be 12.11±0.07, 11.08±0.08, 7.54±0.02, 6.31±0.06 and 15.13±0.05 meq kg^{-1} after 6 months storage, respectively.

From the same Fig, it was noted that the peroxide value of biscuit samples reduced by growing grape pomace extract concentration from 0.5 to 3%. These results suggest the advantage of the antioxidant activity of GPE over artificial antioxidants. The data also indicate that the presence of grape pomace extracts in biscuits was capable of delaying the accumulation of peroxides in the lipid, comparable to the findings of Reddy, Urooj, and Kumar (2005).

3.6.5. Free fatty acid (FFA)

The FFA parameter is also a measure of the food rancidity. In the present study, it could be observed that the grape pomace extract was the most efficient in decreasing the FFA value (from 1.13 to 1.09%) compared to control (1.62%) and BHA sample (1.07%) initially (Figure 1b). FFA rates continuously developed (p <0.05) in all biscuit samples throughout the storage for 6 months at room temperature. The development in FFA value may be attributed to a splitting of double bonds of unsaturated fatty acids as stated by earlier authors (Noor, & Augustin, 1984). The increment rates in the values were diminished with the increase of extracts level from 0.5 to 3%.

3.6.6. Thiobarbituric Acid Test (TBA)

TBA value evaluates the formation of secondary oxidation products, mostly malonaldehyde, which may provide off-flavor to oxidize oil (Rossel, 1994). The impact of BHA and various grape pomace extracts on the TBA content of biscuit samples throughout the storage for 6 months at room temperature is represented in Figure 1c. The addition

of GPE at different levels (0.5 - 3%) resulted in an insignificant decrease (from 0.381 to 0.377 mg malonaldehyde/kg sample) in TBA contents of biscuits when compared with TBA values of control biscuit and the sample enriched BHA (0.385 and 0.383 mg/kg, respectively) at the beginning. These data are harmonious with the findings of Izzreen, and Noriham, (2011) and Ibrahium, El-Ghany, and Ammar, (2013). They observed that the addition of some natural antioxidant extracts to cakes and crackers samples reduced TBA content in all treatments at the beginning and throughout 28 days of storage compared to an artificial antioxidant influence. Contrastingly, it was obvious after 6 months of storage, the additions of GPE at different levels retard the growth of rancidity in biscuits. Furthermore, it could be noted that the GPE with all its levels displayed the greatest protection towards decreasing the TBA contents in biscuits (from 0.563 to 0.377 mg/kg) throughout various storage periods in comparison with the control sample (0.805 mg/kg) and BHA (0.669 mg/kg). Our findings are in agreement with the outcomes achieved by Reddy et al. (2005).

4. CONCLUSIONS

The data of this study demonstrated that GPE is a natural source of phenolic compounds. The methanol was the best solvent for the extraction of phenolic compounds compared to ethanol and acetone. Besides, the obtained results showed that the addition of grape pomace extracts gave an excellent antioxidant impact on the biscuits compared to BHA. Results of the sensory evaluation indicated that the selected grape pomace extracts at levels of 0.5% and 1% might be used instead of synthetic antioxidants since these extracts didn't show dramatic adverse effects on the organoleptic features of the biscuit. Therefore, grape pomace may contain beneficial bioactive components as natural antioxidants and could be valuable in the food industry.

REFERENCES

1. AACC (2000). American Association of Cereal Chemists. Approved Method of the AACC, 10th ed., American Association of Cereal Chemists, St., Paul, Minnesota, USA.
2. Ajila, C. M., Aalami, M., Leelavathi, K., & Rao, U. P. (2010). Mango peel powder: A potential source of antioxidant and dietary fiber in macaroni preparations. *Innovative Food Science & Emerging Technologies*, *11*(1), 219-224.
3. Ajila, C. M., Leelavathi, K. U. J. S., & Rao, U. P. (2008). Improvement of dietary fiber content and antioxidant properties in soft dough biscuits with the incorporation of mango peel powder. *Journal of cereal science*, *48*(2), 319-326.
4. Amendola, D., De Faveri, D. M., & Spigno, G. (2010). Grape marc phenolics: Extraction kinetics, quality and stability of extracts. *Journal of Food Engineering*, *97*(3), 384-392.
5. Antoniolli, A., Fontana, A. R., Piccoli, P., & Bottini, R. (2015). Characterization of polyphenols and evaluation of antioxidant capacity in grape pomace of the cv. Malbec. *Food Chemistry*, *178*, 172-178.
6. AOCS (1996). Official Methods Recommended Practices of the AOCS, 6th ed., Champaign, IL USA. Association of Cereal Chemists, INC. st., Paul, Minnesota, USA.
7. Aziah, N. A. A., & Komathi, C. A. (2009). Acceptability attributes of crackers made from different types of composite flour. *International food research journal*, *16*, 479-482.
8. Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, *239*(1), 70-76.
9. Beres, C., Simas-Tosin, F. F., Cabezudo, I., Freitas, S. P., Iacomini, M., Mellinger-Silva, C., & Cabral, L. M. (2016). Antioxidant dietary fibre recovery from Brazilian Pinot noir grape pomace. *Food Chemistry*, *201*, 145-152.
10. Binaschi, M., Garrido, G. D., Cirelli, C., & Spigno, G. (2018). Biotechnological strategies to valorise grape pomace for food applications. *Chemical Engineering Transactions*, *64*, 367-372.
11. Davidov-Pardo, G., Moreno, M., Arozarena, I., Marín-Arroyo, M. R., Bleibaum, R. N., & Bruhn, C. M. (2012). Sensory and consumer perception of the addition of grape seed extracts in cookies. *Journal of Food Science*, *77*(12), S430-S438.
12. Dumitriu, D., Peinado, R. A., Peinado, J., & De Lerma, N. L. (2015). Grape pomace extract improves the in vitro and in vivo antioxidant

properties of wines from sun light dried Pedro Ximénez grapes. *Journal of Functional Foods*, *17*, 380-387.

13. El-Faham, S. Y., Mohsen, M. S., Ashour, Sharaf, A. M., & Zaky, A. A. (2016). Utilization of Mango Peels as a Source of Polyphenolic Antioxidants. *Current Science International, 05 (04),* 529-542.
14. González-Centeno, M. R., Comas-Serra, F., Femenia, A., Rosselló, C., & Simal, S. (2015). Effect of power ultrasound application on aqueous extraction of phenolic compounds and antioxidant capacity from grape pomace (*Vitis vinifera* L.): experimental kinetics and modeling. *Ultrasonics Sonochemistry*, *22*, 506-514.
15. González-Centeno, M. R., Rosselló, C., Simal, S., Garau, M. C., López, F., & Femenia, A. (2010). Physico-chemical properties of cell wall materials obtained from ten grape varieties and their byproducts: grape pomaces and stems. *LWT-Food Science and Technology*, *43*(10), 1580-1586.
16. Ibrahium, M., El-Ghany, M. A., & Ammar, M. (2013). Effect of clove essential oil as antioxidant and antimicrobial agent on cake shelf life. *World Journal of Dairy & Food Sciences*, *8*(2), 140-146.
17. Izzreen, I., & Noriham, A. (2011). Evaluation of the antioxidant potential of some Malaysian herbal aqueous extracts as compared with synthetic antioxidants and ascorbic acid in cakes. *International Food Research Journal*, *18*, 583-587.
18. Jayaprakasha, G. K., Singh, R. P., & Sakariah, K. K. (2001). Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. *Food Chemistry*, *73*(3), 285-290.
19. Kammerer, D., Claus, A., Carle, R., & Schieber, A. (2004). Polyphenol screening of pomace from red and white grape varieties (*Vitis vinifera* L.) by HPLC-DAD-MS/MS. *Journal of Agricultural and Food Chemistry*, *52*(14), 4360-4367.
20. Lavelli, V., Torri, L., Zeppa, G., Fiori, L., & Spigno, G. (2016). Recovery of winemaking by-products for innovative food application. *Italian Journal of Food Science*, *28*, 542-564.
21. Llobera, A., & Can˜ellas, J. (2007). Dietary fibre content and antioxidant activity of Manto Negro red grape (*Vitis vinifera*): pomace and stem. *Food Chemistry*, *101*, 659–666.
22. Luthria, D. L. (2006). Significance of sample preparation in developing analytical methodologies for accurate estimation of bioactive compounds in functional foods. *Journal of the Science of Food and Agriculture, 86,* 2266-2272.
23. Maner, S., Sharma, A. K., & Banerjee, K. (2017). Wheat flour replacement by wine grape pomace powder positively affects physical, functional and sensory properties of cookies. *Proceedings of*

the National Academy of Sciences, India Section B: Biological Sciences, *87*(1), 109-113.

24. Montealegre, R. R., Peces, R. R., Vozmediano, J. L. C., Gascueña, J. M., & Romero, E. G. (2006). Phenolic compounds in skins and seeds of ten grape Vitis vinifera varieties grown in a warm climate. *Journal of Food Composition and Analysis, 19,* 687–693.
25. Negro, C., Tommasi, L., & Miceli, A. (2003). Phenolic compounds and antioxidant activity from red grape marc extracts. *Bioresourc Technology*, *87*, 41–44.
26. Noor, N., & Augustin, M. A. (1984). Effectiveness of antioxidants on the stability of banana chips. *Journal of the Science of Food and Agriculture*, *35*(7), 805-812.
27. Norhidayah, A., Babji, A. S., Shazali, M. S., Norazmir, M. N., & Norazlanshah, H. (2011). Effects of Mango (*Mangifera indica* L.) and Guava (*Psidium guajava* L.) Extract on Frozen Chicken Meat Balls' Storage Quality. *Pakistan Journal of Nutrition*, *10*(9), 879-883.
28. Oroian, M., & Sorina, R. (2017). Honey authentication based on physicochemical parameters and phenolic compounds. *Computers and Electronics in Agriculture, 138,* 148-156.
29. Pedroza, M. A., Amendola, D., Maggi, L., Zalacain, A., De Faveri, D. M., & Spigno, G. (2015). Microwave-assisted extraction of phenolic compounds from dried waste grape skins. *International journal of food engineering*, *11*(3), 359-370.
30. Pérez-Jiménez, J., Arranz, S., Tabernero, M., Díaz-Rubio, M. E., Serrano, J., Goñi, I., & Saura-Calixto, F. (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food Research International*, *41*(3), 274-285.
31. Pinto, D., Castro, I., Vicente, A., Bourbon, A. I., & Cerqueira, M. A. (2014). Functional Bakery Products-An Overview and Future Perspectives. *Bakery products science and Technology*, 431-452.
32. Reddy, V., Urooj, A., & Kumar, A. (2005). Evaluation of antioxidant activity of some plant extracts and their application in biscuits. *Food Chemistry*, *90*(1-2), 317-321.
33. Rockenbach, I. I., Rodrigues, E., Gonzaga, L. V., Caliari, V., Genovese, M. I., Gonçalves, A. E. D. S. S., & Fett, R. (2011). Phenolic compounds content and antioxidant activity in pomace from selected red grapes (*Vitis vinifera* L. and *Vitis labrusca* L.) widely produced in Brazil. *Food Chemistry*, *127*(1), 174-179.
34. Rowayshed, G., Sharaf, A. M., El-faham, S. Y., Ashour, M., & Zaky, A. A. (2015). Utilization of potato peels extract as source of

phytochemicals in biscuits. *Journal of Basic and Applied Research International*, 190-201.

35. Shahidi, F., & Wanasundara, U. N. (2002). Methods for measuring oxidative rancidity in fats and oils. In *Food Lipids* (pp. 484-507). CRC Press.
36. Wang, X., Chen, H., Fu, X., Li, S., & Wei, J. (2017). A novel antioxidant and ACE inhibitory peptide from rice bran protein: Biochemical characterization and molecular docking study. *LWT-Food Science and Technology, 75,* 93-99.
37. Zaky, A. A., Chen, Z., Liu, Y., Li, S., & Jia, Y. (2019). Preparation and assessment of bioactive extracts having antioxidant activity from rice bran protein hydrolysates. *Journal of Food Measurement and Characterization*, *13*, 2542–2548. https://doi.org/10.1007/s11694-019-00174-9
38. Zhang, Z., Liao, L., Moore, J., Wu, T., & Wang, Z. (2009). Antioxidant phenolic compounds from walnut kernels (*Juglans regia* L.). *Food Chemistry*, *113*, 160–165.

Table 1 Chemical composition of grape pomace waste

Parameters	% dry weight
Moisture*	62.79 ± 0.13
Crude protein	12.26 ± 0.45
Fat	6.07 ± 0.03
Ash	8.49 ± 0.01
Crude fiber	11.58 ± 0.34
Carbohydrates**	61.60

The values are the mean ± SD of three replicates. *on fresh weight basis. **by difference.

Table 2 Extraction yield and total phenolic content of grape pomace extracts

Sample	Solvent	Extraction yield (%)	Total phenol content (mg GAE 100 g^{-1} DW)
Grape pomace	Methanol	7.22 ± 0.08[a]	327.71 ± 0.67[a]
	Ethanol	4.16 ± 0.21[b]	205.53 ± 0.11[b]
	Acetone	2.46 ± 0.53[c]	104.21 ± 0.33[c]

The values are the mean ± SD of three replicates. The values in the same column followed by different letters are significantly different ($p < 0.05$).

Table 3 Antioxidant activities of grape pomace extract

Samples	DPPH (%)	FRAP (mmol Fe_2SO_4/100g)
Grape pomace	86.89 ± 0.75[a]	73.93 ± 0.22[a]
BHA	65.20 ± 0.49[b]	52.46 ± 0.53[b]

The values are the mean ± SD of three replicates. The values in the same column followed by different letters are significantly different ($p < 0.05$). ***DPPH*** radical scavenging assay; ***FRAP*** ferric-reducing antioxidant power.

Table 4 Identification of phenolic compounds of grape pomace extract using HPLC analysis

Individual phenolic acids	Concentration (mg/100g)
Gallic acid	11.43 ± 1.23
Caffeic acid	3.29 ± 2.45
Sinapic acid	nd
Chlorogenic acid	6.44 ± 1.16
Syringic acid	nd
Ferulic acid	nd
Isoferulic acid	nd
Protocatechuic acid	13.91 ± 3.22
Vanillic acid	6.00 ± 1.27
Isovanillic acid	4.07 ± 2.56
Tyrosol	nd

Quercetin	16.43 ± 0.57
Apigenin	nd
Catechin	38.94 ± 0.17

The values are the mean ± SD of three replicates. nd= not detected.

Table 5 Influence of grape pomace extracts with different levels on the physical measurements of biscuits

Treatments	Diameter W (cm)	Thickness T (cm)	Spread ratio W/T
Control	6.86±0.04^{d}	0.89±0.01^{a}	7.97^{d}
BHA	6.76±0.03^{c}	0.80±0.09^{c}	8.45^{c}
GPE (%)			
0.5	7.03±0.13^{b}	0.83±0.03^{b}	8.46^{c}
1	7.06±0.07^{b}	0.76±0.04^{d}	9.28^{b}
2	7.06±0.09^{b}	0.76±0.04^{d}	9.28^{b}
3	7.09±0.04^{a}	0.70±0.06^{e}	10.12^{a}

The values are the mean ± SD of three replicates. The values in the same column followed by different letters are significantly different ($p < 0.05$). ***GPE*** grape pomace extract.

Table 6 Influence of grape pomace extracts with different levels on the color of biscuits

Treatments	L^*	a^*	b^*
Control	63.58±2.64^{a}	14.04±0.01^{a}	25.41±0.26^{a}
BHA	56.57±0.02^{c}	11.85±1.17^{b}	24.5±0.01^{c}
GPE (%)			
0.5	63.33±.55^{a}	8.79±0.64^{e}	24.94±0.43^{b}
1	58.71±.89^{b}	10.72±0.76^{c}	23.51±0.25^{d}
2	56.35±1.61^{c}	10.36±0.27^{cd}	22.88±0.33^{e}
3	52.65±0.44^{d}	10.11±0.28^{d}	22.51±0.42^{f}

The values are the mean ± SD of three replicates. The values in the same column followed by different letters are significantly different ($p < 0.05$). ***GPE*** grape pomace extract. ***L*** lightness, **a*** redness, **b*** yellowness.*

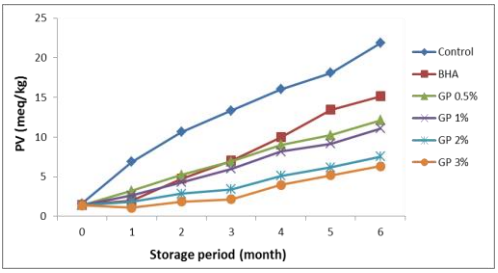
Table 7 Sensory evaluation of biscuits incorporated with grape pomace extracts

Treatments	Color	Odor	Taste	Texture	Overall quality
Control	7.8±1.37^{a}	7.13±1.40 ^{a}	7.2±1.82 ^{a}	7.13±1.76 ^{a}	7.93±1.22^{a}
BHA	6.46±1.84^{b}	6.00±1.92^{b}	5.73±2.52^{b}	6.66±1.75^{b}	6.26±2.25^{b}
GPE (%)					
0.5	6.56 ±1.35^{ab}	6.06 ±2.14^{ab}	5.8 ±1.12^{ab}	6.53 ±1.53^{ab}	6.30 ±2.57^{ab}
1	6.08 ±1.72^{ab}	5.66 ±2.12^{c}	5.20 ±1.05^{ab}	6.26 ±1.33^{ab}	6.06 ±2.18^{ab}
2	5.8 ±1.19^{c}	5.00 ±1.98^{d}	5.33±1.24 ^{c}	5.86 ±1.12^{c}	5.20±2.26^{c}
3	5.46±1.17^{d}	4.53 ±2.09^{e}	4.53 ±1.19^{d}	5.46 ±1.22^{d}	4.73±1.73^{d}

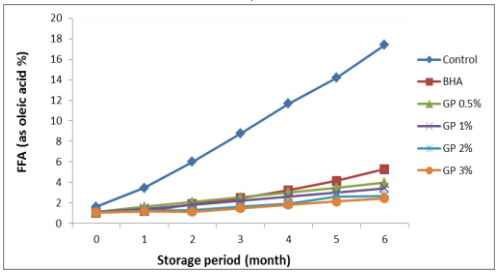
The values are the mean ± SD of three replicates. The values in the same column followed by different letters are significantly different ($p < 0.05$). ***GPE*** grape pomace extract.

Figure Captions

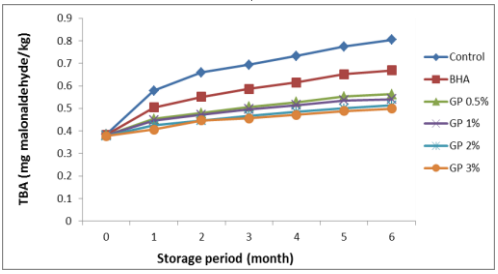
Figure 1. Effect of BHA and grape pomace extracts on (a) peroxide value (PV) and (b) free fatty acid (FFA) and (c) thiobarbituric acid (TBA) of stored biscuits for 6 months. Values are mean ± SD (n = 3).



a)



b)



c)