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Antioxidant Activity of Apple Peels Bioactive Molecules Extractives

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Abstract In the present work apple peel extracts of (Total Phenols, Flavonoids, and Anthocyanin) compounds from the fruits cultivated in Kurdistan Region of Iraq (BarwariBala mountains in Duhok governerate) are prepared. The three extracts are evaluated concerning the yield and the antioxidant activity of the bioactive compounds by four methods (β-Carotene-linoleic acid assay, reducing power assay, scavenging of hydrogen peroxide, and total antioxidant capacity). The factors affecting antioxidant activity including temperature, pH and incubation time were optimized using Response Surface Methodology. The effect of storage time on antioxidant activity was also studied. The results confirmed that the yield of total phenols were 23.33%, about 31.20 % of the total phenols were flavonoids, while anthocyanin compounds formed 9.76% (g/g raw material). The three extracts showed antioxidant activities of different extents. Response surface analysis results revealed that increasing the incubation time and temperature resulted in decrease antioxidant capacity; however, temperature seemed of top most significant compared to incubation time. The antioxidant capacity of the extracts showed gradual decrease with increasing pH up to pH= 9, followed by an increase towards high pH values. The antioxidant capacity of all extracts were gradually reduced with increasing storage time. The antioxidant capacity of anthocyanin extract was slightly affected by storage time compared to alcoholic and flavonoids extracts. The overall results confirm that the high content of phenolic compounds and antioxidant activity of apple peels indicates that they should be regarded as source of antioxidants.

Keywords Antioxidant activity, Apple peel, Response Surface Methodology

1. Introduction

A characteristic of higher plants is the production of a wide variety of natural products, called secondary metabolites; the majority of them have economical and industrial importance drugs, flavor and fragrance, dye and pigments, pesticides, and food additives. Research on natural products with potential health benefits represents an area of great interest in which fruits had been the most important source.

Apple (*Malus domestica*) of the rose family (Rosaceae) is a delicious fruit and is the fourth most widely produced fruit worldwide (Konarska, 2013). Apple has got high medicinal value; the pulp, the seeds and the peels possess medicinal property (Balasuriya and Rupasinghe, 2012; Ismail et al., 2012; Otakar et al., 2012; Thilakarathna, and Rupasinghe, 2013). There is a great abundance of literature data concerning the medical benefits of apple are such as antioxidative, anticancer, and antimutagenic efficacy (Boyer and Liu, 2004; Rapasingle et al., 2011; Giomaro et al., 2014). Moreover, as compared with some other core fruit species (e.g., pears), apples contain less energy and show a high content of minerals, pectins and vitamin C (Rapasingle et al., 2011).

Apple peel as by-product normally is directly disposed as waste. The results from previous studies showed that the peel has high content of phenolic compounds, antioxidant activity, and antiproliferative activity (Al-Zoreky 2009; Balasuriya and Rupasinghe 2012; Woife et al., 2003; Huber and Rupasinghe; 2009; He and Liu, 2008; Massini et al., 2013). Furthermore, Many studies revealed that independently of apple varieties and other circumstances, peel contains more phenolics than the flesh (Kalinowska et al., 2014; Drogoudi et al., 2008; Kalinowska et al., 2012).

In general, the antioxidants are beneficial because they neutralize free radicals that can damage cells and tissues. The most widely used synthetic antioxidants in food (butylatedhydroxytoluene BHT, butylatedhydroxyanisole BHA, and tertiary butyl hydroquinone TBHQ) have been suspected to cause or promote negative health effects (Pokorny, 2007; Yoshihara et al., 2010). For this reason there is a growing interest in studies of natural additives as potential antioxidants for several industrial applications,

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mainly concerning the cosmetic, and the food preservation and formulation (Mendiola et al., 2010).

The antioxidants level is significantly influenced by the apparatuses used in the extraction, the nature of the extraction solvent, extraction time and temperature, as well as the interaction between these factors. However, it was reported that solvent extracting power is the most important factor affecting antioxidant capacity (Jin et al., 2015; Dorta et al., 2013) followed by the apparatuses that are used in the extraction (Zang et al., 2014).

On the other hand, the antioxidant activities is highly affected by storage conditions. Most of the studies carried out in concern confirmed that antioxidant activity decreases during storage. The reason may be attributed to dilution of antioxidant components by increased moisture and also to possible oxidation of antioxidant components (Dar and Nayik, 2016). The exposure to heat and light during storage could change or degrade the structure of the antioxidants, resulting in marked changes in their affinity (Fracassetti et al., 2013; Del-Toro-Sánchez et al., 2015).

A number of studies have been carried out on the phenolic compounds and antioxidant In Kurdistan region of Iraq, there are many members of the genus Malus that are used in diet, thus, the main aim of the work described here is to focus on the fruit waste; the peel of typical native apple cultivars and to determine the basic chemical and biological characteristics which are incompletely known such as the activities of the different bioactive molecules in order to improve knowledge of apple peels as raw material for antioxidant production, and to contribute to their impact on the management of a variety of clinical conditions and maintenance of health.

2. Experimental

2.1. Raw Material

Apple fruit was collected from Kurdistan Region of Iraq (BarwariBala Duhok mountains). The fruits were peeled and the fresh peels were extracted after minced to very fine pieces.

2.2. Chemicals and Reagents

All the used solvents and chemicals were of analytical grade produced from Sigma Aldrich.

2.3. Preparation of Plant Extracts

2.3.1. Extraction of total Phenol Content (alcoholic extract)

The alcoholic extract was prepared following the procedure of (Laleh et al., 2006; Banso, 2009).Thirty g of the fresh peels were mixed with 200 ml (70%) ethanol using electrical blender and the mixture was shaken in thermostatic water bath at 40 ℃ for 4 h. The residue was removed by filtration using filter paper (Whattman No. 40) and the filtrate was concentrated by evaporation using rotary evaporator to afford (7.00 g) of extract.

2.3.2. Flavonoids Extract

The flavonoid extract was prepared following literature procedure (Peach et al., 1955; Harborne, 1984; Andersen and Markham, 2006). Five g of alcoholic extract were dissolved in (100 ml) distilled water then 125 ml of (1%) aqueous lead acetate was added with stirring for 5 min. The solution was left for 2 h to complete the precipitation. The solution was filtered using filter (Whattman No. 40) and the filtrate was concentrated by rotary evaporator.

The resulted semi-solid material was then dissolved in absolute methanol. Fifty ml of (1%) methanolic lead acetate was added with stirring for few min and the solution was left for 2 h for precipitation completion. The mixture was filtrated using Buchner funnel and the precipitate was washed once with 10 ml distilled water, then with 10 ml absolute methanol and finally washed for 3 times with 10 ml ethyl acetate.

The precipitate was left to dry in dark at room temperature. The dry precipitate was dissolved in [25 ml acetone + 5 ml (2M HCl)] with shaking, and then left till the complete precipitation of white precipitate. The supernatant was separated and evaporated under vacuum at 40 ℃ to afford (1.56 g) extract which referred as flavonoid extract.

2.3.3. Anthocyanin Extract

The anthocyanin extract was prepared following the procedure mentioned by (Harborne, 1984; Schofs, 2004; Andersen and Markham, 2006). Twenty five g of the fresh peels were mixed for 5 min with 50 ml of acidified methanol (1% HCl in methanol) using electrical blender. The mixture was transferred to amber conical flask and placed in an ice bath and stirred for 12 h.

The mixture was filtrated by Buchner funnel and the filtrate was collected and placed in dark at room temperature till dryness. The dry material was washed twice with (25 ml) acidified methanol and filtered to obtain (2.44 g) extract which referred as anthocyanin extract.

2.4. Antioxidant Activity of the Extracts

The antioxidant activity for the 3 extracts was evaluated by 4 methods:

2.4.1. β-Carotene- Linoleic Acid Assay

In β -carotene-linoleic acid assay, the antioxidant activity (AA) of the 3 apple peel extracts were compared with antioxidant activity of a synthetic antioxidants (α-tocopherol) during subjected to thermal autoxidation at 50 ℃ following the procedure of (Marco, 1968). The mechanism of bleaching of β-carotene is a free-radical-mediated phenomenon resulting from the hydroperoxides formed from linolic acid. β-carotene, in this model system, undergoes rapid discoloration in the absence of an antioxidant.

The linoleic acid free radicals, formed upon the abstraction of hydrogen atom from one of its diallylic

methylene groups, attacks the highly unsaturated β-carotene molecules. As β-carotene molecules lose their double bonds by oxidation, the compound loses its chromophore and characteristic orange color, which can be monitored spectrophotometrically.

The presence of different extracts can hinder the extent of β-carotene-bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system. The values of AA are tabulated in Table (2), and plotted as shown in Figure (1).

2.4.2. Reducing Power Assay

The ability of alcoholic, flavonoids, and anthocyanin extracts of apple peels to reduce iron (III) to iron (II) was determined according to the procedure of (Oyaizu, 1988; Yildirim et al., 2001; Tsasi et al., 2006; Su et al., 2009) and compared to that of ascorbic acid, which is known to be a strong reducing agent. The % reduction (R.P. %) of the samples was plotted against concentration as shown in Figure (2).

2.4.3. Scavenging of Hydrogen Peroxide

The ability of alcoholic, flavonoids, and anthocyanin extracts of apple peels to scavenge H_2O_2 was determined following the methods of (Ruch et al., 1989; Oktay et al., 2003) using the calibration curve between concentration and absorbance at 230 nm of standard ascorbic acid. The results of the test are shown in Table (3).

2.4.4. Total Antioxidant Capacity

The test is based on the reduction of Mo (VI) to Mo (V) by the samples of alcoholic, flavonoids, and anthocyanin extracts of apple peels. The antioxidant capacity of the extracts was evaluated following a literature method of (Prieto et al., 1999 and Deloueeet al., 2007) and expressed as micromoles of α-tocopherol equivalent per milliliter of extracts using the calibration curve of α-tocopherolat 695 nm. The concentration was determined from Beer's Law using extinction coefficient of (4 x 103 M^{-1} cm^{-1}). The results of the test are shown Figure (3).

3. The Impact of Temperature, pH, and Heating Time on the Antioxidant Activity

3.1. Statistical Analysis Using Response Surface Methodology (RSM)

Response surface methodology (RSM) in conjunction with central composite rotatable design (CCRD) was performed in the present study after preliminary experiments. The RSM was used for optimization the impact of temperature, pH, and heating time on antioxidant activity for the 3 extracts.

RSM was introduced by Box and Wilson in 1951 (Box and Wilson, 1992). The methodology is commonly used for the experimental design (Elaydi et al., 2014). The RSM normally allow identifying the effects of operating parameters (independent variables Xi) on different responses Yj (dependent variables).

The statistical analysis procedure of statgraphics plus for windows software (5.1 version) for experimental design and data treatment was used and a central composite design consisting of 18 experimental runs with 4 replicates at the center point was established. The mathematical empirical model applies in this study was:

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i \chi_i + \sum_{i=1}^{n} \beta_{ii} \chi_i^2 + \sum_{i=1}^{n-1} \sum_{j=2}^{n} \beta_{ij} \chi_i \chi_j + \varepsilon$$

where Y: is the response or dependent variable; $\chi_i$and χ_j: the independent variables; i and j: the indices; β_0, β_i, β_{ii}, β_{ij}: the regression coefficients; and ε: is the random error of the factors.

The analyses of variance (ANOVA) were used to determine significant differences between the independent variables ($p<0.05$). Pareto chart was used to identify the impact of variables on various responses. The vertical line in the Pareto chart determines the effects that are statistically significant at 95% as confidence level. Surface methodology plots were used to optimize the various responses. The levels of independent variables used in experimental design are listed in Table 1which shows the independent variables and their levels. α the (axial distance) = $\sqrt[4]{2^k}$, k is the number of orthogonal design variables (in the present case, k=3).

Table 1. Levels of independent variables used in experimental design

Coded level	-α	-1	0	+1	+α
Temperature ℃	23	40	65	90	107
pH	2	4	7	10	12
Incubation time (min.)	36.5	55	82.5	110	129

3.2. Determination of the Dependent Variable (The Antioxidant Activity: AA)

The residual antioxidant capacity of alcoholic, flavonoids, and anthocyanin extracts of apple peels were determined for the extracts treated according to the experiments established by RSM. The response values are listed in Table 2.

The (AA) were expressed as µ of α-tocopherol equivalent per ml of extracts using the calibration curve of α-tocopherol at 695 nm. The concentration was determined from Beer's law using extinction coefficient of (4 x 103 M^{-1} cm^{-1}). The response analysis for each experiment was replicated 3 times, and the average value was recorded.

Table 2. Independent variables and their coded and natural levels employed in a central composite design for optimization of apple peels extracts

Exp. No.	Temp. ℃	Incubation time (min.)	pH	AA Alcohol extract µmole/ml	AA Flavonoids extract µmole/ml	AA Anthocyanin extract µmole/ml
4 central points	65	82.5	7	125 ±1.9	135 ±2.3	120 ±1.6
1	90	55	4	90.24	89.76	88.08
2	107	82.5	7	50.03	60.35	58.97
3	40	55	4	155.06	154.98	151.89
4	40	55	10	169.85	170.01	163.98
5	90	110	10	70.08	70.21	68.86
6	65	82.5	2	160.07	160.43	150.11
7	65	36	7	150.14	145.23	142.09
8	23	82.5	7	150.08	149.87	147.07
9	90	55	10	90.21	80.16	77.09
10	90	110	4	75.33	80.13	80.45
11	65	82.5	12	130.29	140.31	145.24
12	40	110	10	140.11	139.99	140.87
13	65	129	7	118.22	117.96	120.21
14	40	110	4	150.08	150.25	147.32

Data presented as mean ±SD of 3 replicates.

Table 3. Yield of bioactive molecules and antioxidant activity average values of alcoholic, flavonoids, and anthocyanin extracts of apple peels (conc. = 0.005 µg/ml) based on different test methods

Sample	Yield of bioactive molecules %	β-Carotene-linoleic acid assay % AA	Reducing power assay R.P. %	Scavenging of hydrogen peroxide (mg AAE/g) eq.	Total antioxidant capacity (µmole/ml) eq.
Alcoholic extract	23.33	54.07	49	140.10	185.15
Flavonoids extract	31.20 (In alcoholic extract)	92.01	28	200.23	170.23
Anthocyan extract	9.76 (In peels)	55.80	32	222.05	160.36
Synthetic antioxidant	-	80.90	75	195.27	190.21

4. Results and Discussion

Phenolic compounds are one class of antioxidant agents which act as good proton donors resulting in free radical terminators and contributed to the antioxidant activities of plant (Garzn and Wrolstad, 2009). Flavonoids are groups of polyphenolic compounds, which exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic, antiulcer, anti-allergic, anti-viral, and anticancer activities.

They also act as inhibitors enzymes such as reducates and xanthine oxidase. They are capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups and are potent antioxidant (Su et al., 2009). Escarpa et al., 1998 and Wolfe et al., 2003 and other researchers reported that apple peels possess high contents of phenolic compounds compared to other edible parts of the apple. The fact suggests that apple peels may possess more bioactivity than the flesh. It was also reported that the anthocyanin content of apple peels is related to their appearance. The red color of apple peels is due to the presence of cyanidin 3-galactoside (Awad et al., 2000).

The yield of the bioactive molecules, and the evaluation results of AA of the 3 extracts and the synthetic antioxidant samples of identical concentrations tested by different methods are shown in Table 3.

According to the preparation methods of the extracts, the yield of the bioactive molecules (total polyphenols) extracted by ethanol from the apple peels were 23.33% (g/g raw material), about 31.20% of the total phenols were Flavonoids, while anthocyanin compounds extracted from the waste peels formed 9.76%.

The results of AA showed that the flavonoids extract exhibited the highest AA by β-Carotene- linoleic acid assay, and the anthocyanin extract showed the highest AA by scavenging of hydrogen peroxide test compared to the synthetic antioxidants and the 2 other extracts. However, the 3 apple peels extracts seemed to exhibit lower antioxidant activity measured by reducing power assay and total antioxidant capacity compared to the synthetic antioxidants.

The extracts of high AA reflected that they compose compounds of high degree of hydroxylation and methoxylation on the aromatic rings (Lachman et al., 2009). Regarding the effects of flavonoids composition on AA, it has been reviewed that the antioxidant activity of flavonoids is closely related to their chemical structure; the number and position of the hydroxyl groups, the presence of glycosides as well as the degree of conjugation of the whole molecule (Apaket al., 2007).

The obtained results were in agreement with those reported by (Wolfe et al., 2003) who stated that the major phytochemicals responsible for antioxidant activity in apple peels are flavonoids, especially rutin, quercetin, isoquercetin and luteolin. Positive correlation between total phenol and antioxidant activity was indicated by many researchers (Brown et al., 2005; Yang et al., 2009; Garzon and Wrolstand, 2009).

On the other hand, In β-carotene bleaching test, a possible explanation for the pro-oxidant properties found in this study may be the formation of highly reactive substances from the extracts which subsequently induced hydroperoxide production in the linoleic acid and ultimately resulted in the oxidation of highly unsaturated and orange-colored β-carotene molecules to colorless compounds. The β-carotene bleaching behavior of the synthetic antioxidant and the extracts are presented in Figure 1.

The bleaching rate of the alcoholic and anthocyanin extracts seemed to be higher than that of synthetic antioxidant in particular during the initial 30 min, while the bleachingrate values of the flavonoids extracts were very close to that for the synthetic anti-oxidant.

The results confirmed that the AA of the anthocyanin and alcoholic extracts of apple peels are approximately 2 times lower than that of α-tocopherol after 120 min, while the AA of the flavonoids extracts was approximated to that of the synthetic anti-oxidant.

The role of reducing power of apple peel extracts on ferric to ferrous reduction in the presence of Fe (II) – stabilizing ligand was investigated and compared to that of ascorbic acid as a strong reducing agent. The results are shown in Figure (2).

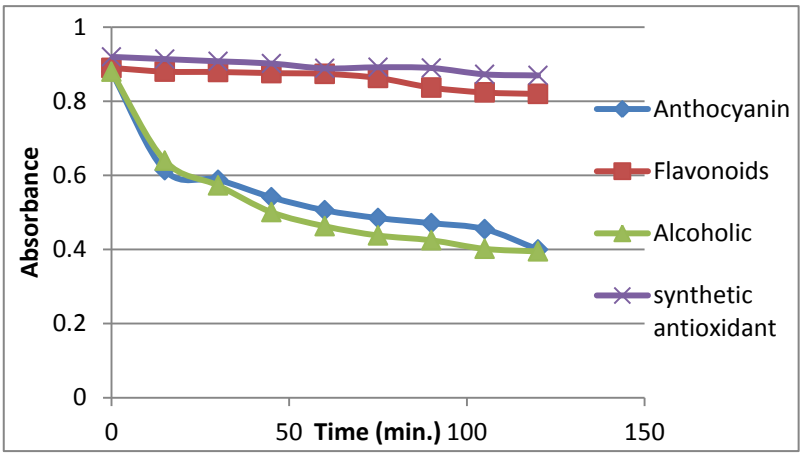

Figure 1. Antioxidant activity of alcoholic, flavonoids, and anthocyanin extracts of apple peels compared to α-tocopherol

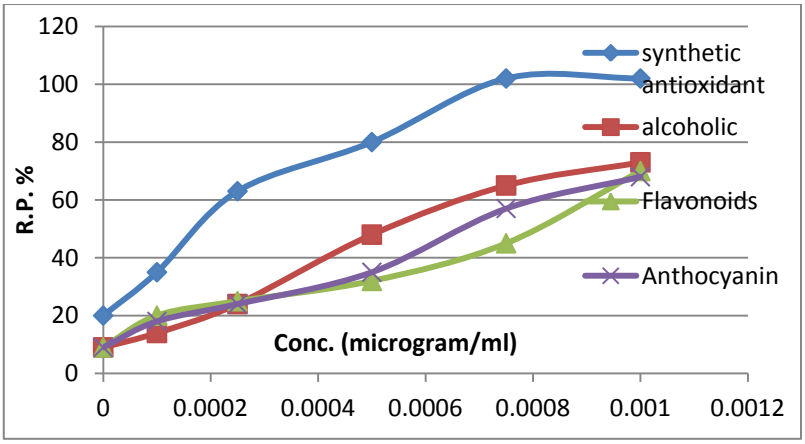

Figure 2. Reducing power of alcoholic, flavonoids, and anthocyanin extract of apple peels as a strong reducing agent compared to Ascorbic acid

The reducing power might be due to the hydrogen donating ability (Akter et al., 2008) and generally associated with the presence of reductants. The extracts have the ability to reduce [Fe $(CN)_6]^{3-}$ to $[Fe(CN)_6]^{4-}$ which consequently reacts with Fe^{3+} and give rise to Prussian blue colored complex $Fe_4[Fe(CN)_6]_3$ (Kaur and Arora, 2008).

The data illustrated in Figure 2 showed that the 3 extracts possess some degree of hydrogen donation capacity in a concentration dependent manner. The reducing power was increased with increasing concentration, but the capacities were inferior to that of ascorbic acid. However, the 3 extracts showed close values to that of ascorbic acid at lower concentrations.

The alcoholic extract was the most potent reducing agent followed by anthocyanin and flavonoids extract. The result reflects that the extracts are of high concentration of phenolic compounds. Phenols that have more number of hydrolysable groups (OH groups) attached to the ring are powerful reducing agent (proton donors) resulting in the termination of free radicals chain reactions (Kaur and Arora, 2008). Nevertheless, at high concentrations, the three extracts showed similar potential proton donor activities.

Hydrogen peroxide is an intercellular precursor of hydroxyl radicals which is very toxic to the cell. It can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups (Swaran, 2009). Hydrogen peroxide can cross cell membranes rapidly and once it is inside the cell, it can probably react with Fe^{+2} and possibly with Cu^{+2} to form hydroxyl radicals. This reaction may be the origin of many toxic effect.

Since antioxidant compounds present in apple peels are good electron donors, they may accelerate the conversion of H_2O_2 to H_2O (Kim, 2013).The results of AA tested by scavenging of hydrogen peroxide method are summarized in Table 3. The results listed in Table 1 confirmed that the strongest anti H_2O_2 activity was observed for anthocyanin extract (222.05 mg AAE/g) followed by flavonoids extract (200.23 mg AAE/g), while the alcoholic extract exhibited the weaker scavenger H_2O_2 activity (140.10 mg AAE/g). The good H_2O_2 scavenger activity is attributed to the presence of phenolic groups that could donate electrons to hydrogen peroxide and neutralizing it into water. Total antioxidant capacity assay was quantified and the results are shown in Figure 3.

Figure 3 illustrates that the flavonoids extract significantly exhibited the highest degree of antioxidant capacity at different concentrations especially at the intermediate concentration (0.0003 µg /ml) followed by ascorbic acid, alcoholic, and anthocyanin extracts.

The situation is also due to the presence of phytochemicals (Banso, 2009). The presence of phenolic compounds which have redox properties allowed them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They may also had a metal chelating potential (Javanmardi et al., 2003).

The literature approved that temperature is one of the important factors that influence the antioxidant capacity (Reblova Z., 2012 and Su et al., 2009). The impacts of temperature, incubation time, and pH of the extracts on AA capacity was investigated and optimized statistically by Response Surface Methodology (RSM). The response surface analysis results for optimization the effect of temperature, incubation time and pH is shown in Figure 4 (A,B,C) for the flavonoids, anthocyanin and alcoholic extracts, respectively.

Figure 4 showes that strong temperature effect and incubation time related decreases in antioxidant capacity. The situation is well clarified by the general trends plots. It could noticed that when the extracts of apple peels are incubated at (23-107 ℃), and when incubation time was extended from 36 to 129 min, the antioxidant capacity of the 3 extracts significantly decreased. However, temperature seemed of top most significant in depression of the antioxidant capacity for the 3 extracts compared to incubation time as clearly reflected by the Pareto charts.

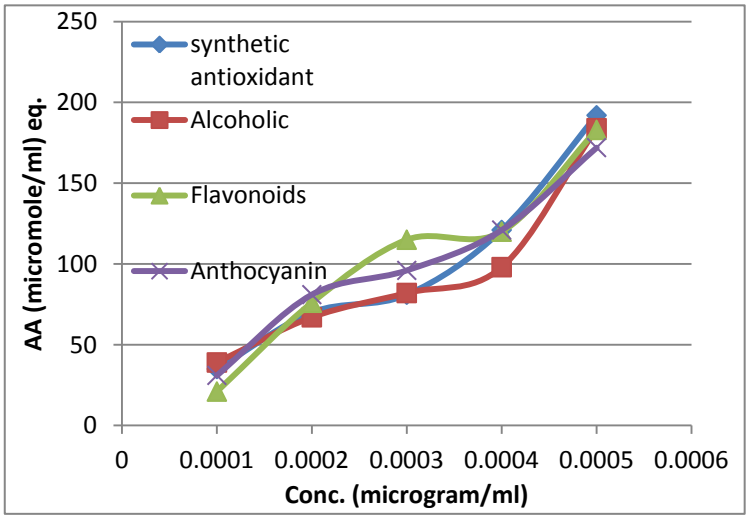

Figure 3. Total antioxidant capacity of alcoholic, flavonoids, and anthocyanin extracts of apple peels compared to the synthetic antioxidant

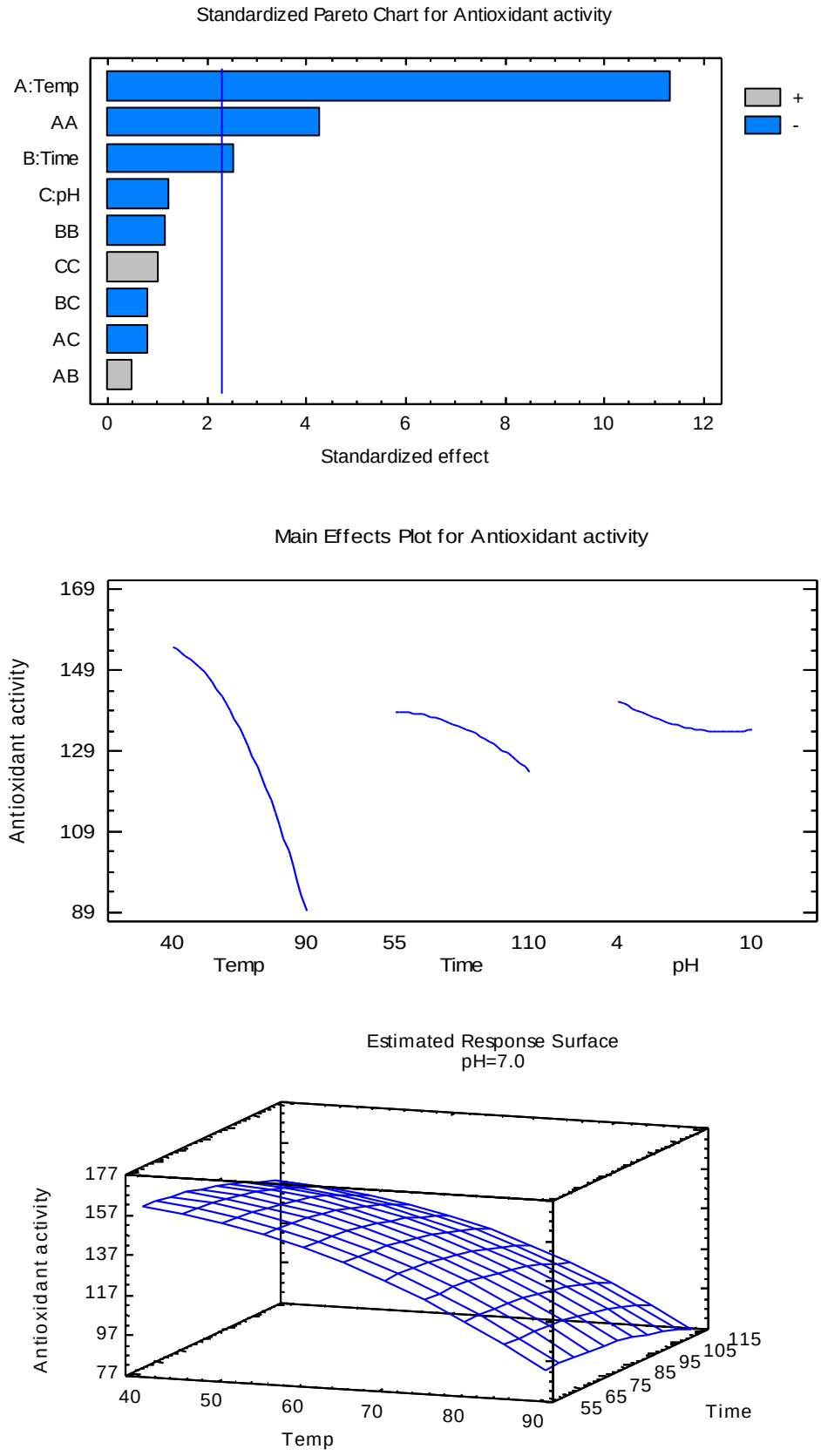

Figure 4(a). Pareto chart, general trends, and response surface of the effects of temp., time and pH on AA for flavonoids extract

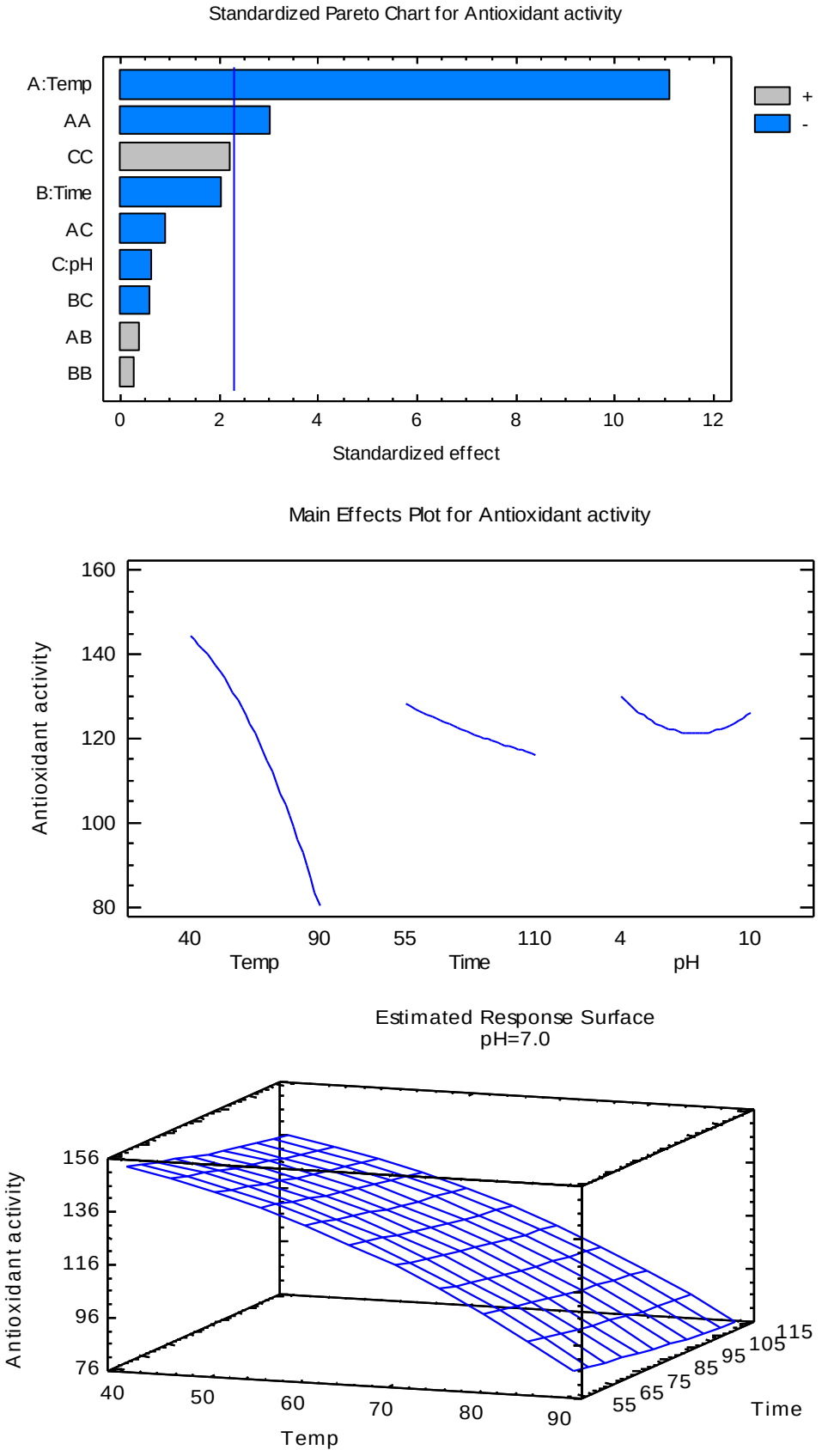

Figure 4(b). Pareto chart, general trends, and response surface of the effects of temp., time and pH on AA for anthocyanin extract

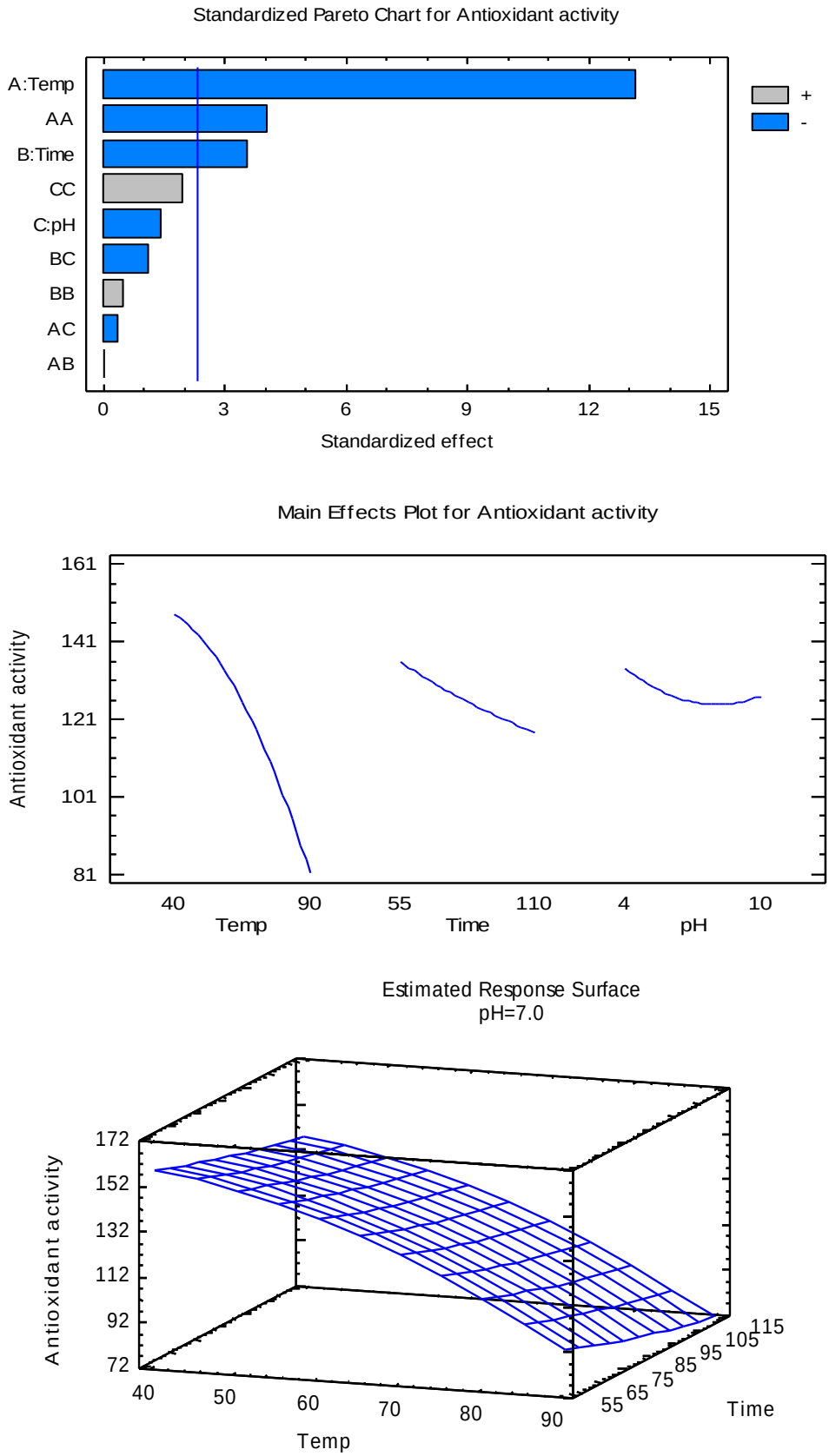

Figure 4(c). Pareto chart, general trends, and response surface of the effects of temp., time and pH on AA for alcoholic Extract

Nevertheless, it is remarkable that the relative rate of the decrease in antioxidant capacity with increasing temperature and incubation time was not the same for the 3 extracts. More noticeable difference between the rates of AA depression has been observed for incubation time parameter. However, the anthocyanin extract showed less significant time effect compared to alcoholic and flavonoids extracts. The decrease in antioxidant capacity may be attributed to the loss of naturally occurring antioxidants or phytochemicals.

Regarding the effect of pH of apple peel extracts on antioxidant capacity, it was noticed that pH had less significant impact on antioxidant capacity of the extracts. An exception was found for the anthocyanin extract {Figure 4 (B)} which showed more significant effect. However, the antioxidant capacity of all the extracts decreased gradually with increasing pH up to pH= 9, followed by an increase towards high pH values.The overall antioxidant activities of the extracts depend on hydrogen-donating ability of phenolic compounds and the stability of phenoxyl radicals formed after the dehydrogenation (Ma et al., 2011).

The antioxidant activity was found to be related to the compound structure such as it is dependable on the number of the included OH group. The more active compound is the more included active groups. The position of the active groups also plays an important role of structure–antioxidant relationship activity (Bendary et al., 2013). The Ortho position was reported to be the more active one, due to its ability to form intra-molecular hydrogen bonding, followed by Para position and then Meta position of compounds. By another word, the increase in antioxidant capacity at high pH is related to hydroxyl moiety deprotonation, resulting in an increase of the antioxidant potential upon formation of the deprotonated forms. The radical scavenging capacity increases because electron-, not H•-, donation becomes easier (Lemanska et al., 2001).

The polynomial empirical models, regression coefficients R2, and the optimized values of AA estimated from response surface analysis (RSA) for the 3 extracts are shown in Table 4.

The high values of R^2 reflect the models fitting to the experimental results. RSM results in Table 4 showed that optimized AA results for the 3 extracts could be achieved when applying the optimized values of temperature, incubation time and pH mentioned in the last left column corresponding to the 3 extracts.

On the another hand, various factors such as cultivation methods, industrial processing, and storage may affect the final concentrations of phytochemicals in food plants and their ventual bio activity. It was reported that phenolic metabolism in apple peel is relatively stable, and the health benefits of phenolics in apple peel is maintained during long-term storage (Golding et al., 2001). In contrast, It is expected that crop cultivars with a higher antioxidant ability have better storage characteristics (Lala, 2008). The effect of storage time at definite storage temperature (5 ℃) on the stability of apple peel extracts is shown in Figure (5).

Table 4. The polynomial empirical model, R^2 (adjusted to d.f.) and the optimized values of AA estimated from RSA for the 3 extracts

Extract type	The polynomial empirical model estimated from RSA	R^2 adjusted	Optimized AA µmole/ml eq.
Alcoholic	$AA=181.104+1.02T-0.28t-4.4P-0.017T^2+0.0Tt-0.017TP+0.0017t^2-0.045tP+0.57P^2$	92.37 %	186.35 at 30 ℃, 36 min., and pH=11
Flavonoids	$AA=122.214+1.43T+0.58t-0.1P-0.02T^2+0.003Tt-0.042TP-0.005t^2-0.038tP+0.34P^2$	89.60 %	181.61 at 25 ℃, 36 min., and pH=12
Anthocyanin	$AA=184.326+0.75T-0.35t-5.7P-0.015T^2+0.002Tt-0.047TP+0.001t^2-0.027tP+0.74P^2$	89.08 %	190.99 at 23 ℃, 50 min., and pH=12

Where T= Temperature, t= time, and P= pH.

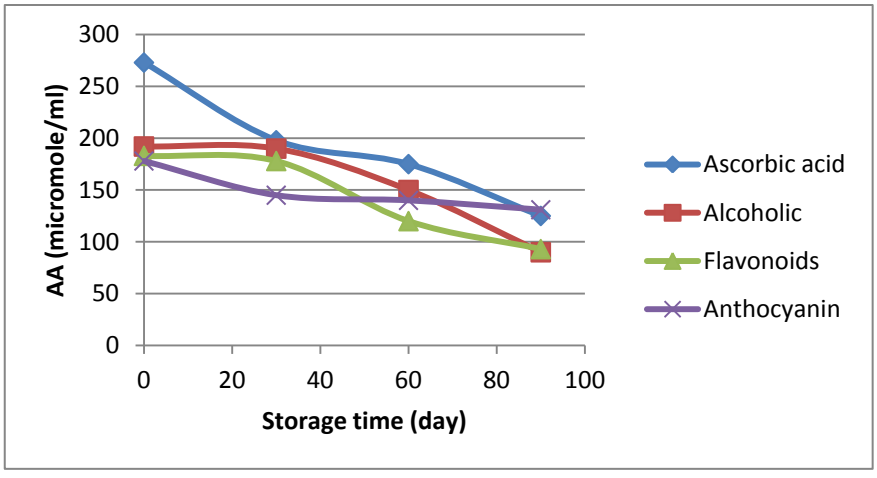

Figure (5). Effect of storage time on the antioxidant capacity of alcoholic, flavonoids, and anthocyanin extracts of apple peels

The extracts were stored in a refrigerator, and the AA was inspected over 90 days. The antioxidant capacity of all the extracts seemed to be reduced gradually along the 90 days period. The antioxidant capacity of anthocyanin extract was slightly affected by the storage conditions applied in this study compared to alcoholic and flavonoids extracts.

The % decrease in AA after 90 day was about 26.5, 49, 53, and 54 % for anthocyanin, flavonoid, alcoholic, and ascorbic acid, respectively. These results were in agreement with the results of (Lala, 2008) and the results on anthocyanin extracts reports by (Arabshahi et al., 2007).

5. Conclusions

Polyphonols are bioactive molecules which are very essential in our life as multifunctional health benefits ingredients. Extraction of these components from different natural resources including fruit waste contribute highly to the economics of industrial production and human needs.

Peels of apples cultivated in Kurdistan region of Iraq (barware mountains) seemed to be rich natural sources for bioactive molecules which reflected the good antioxidant characteristics of the extracts. Response Surface Methodology seemed as an efficient tool to optimize the variables affecting the chemical activity of the extracted value-added compounds.

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