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Antioxidant and Mineral Content of Beverages Based on Permeate with Fruit Juice and Green Tea Extract

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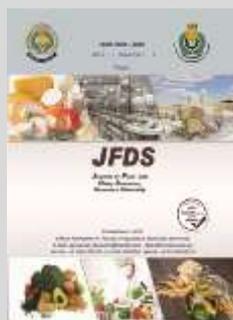


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ABSTRACT

Different fruits (banana, pomegranate, orange and strawberry) permeate beverage enriched with green tea extract were prepared. The sensory characteristics, total soluble solids, pH, antioxidant, minerals (Ca, K, Mg, and Na) and ascorbic acid contents of these beverages were evaluated. Phenolic compounds of green tea were determined by HPLC. Gallic acid (5724.21 µg/g) was the highest value followed by catechin, rutin, cinnamic acid and caffeic acid (3273.30, 239.823, 223.65 and 150.44 µg/g, respectively) and the lowest value of phenolic compound is chlorogenic acid (2.50 µg/g). There are significant differences between all treatments for minerals content. The highest percentage (P<0.05) was for sample B₄ (strawberry permeate beverage enriched with green tea extract) (872.47 µg TE/g) followed by B₂ (Permeate pomegranate green tea extract beverage) (724.21 µg TE/g), B₃ (orange permeate beverage enriched with green tea extract) (568.74 µg TE/g) and the lowest value was (93.79 µg TE/g) for B₁ (Permeate banana green tea extract beverage). The highest value of ascorbic acid was for B₃ (orange permeate beverage enriched with green tea extract) 17.20 mg/L and the lowest value was for B₄ (Permeate strawberry green tea extract beverage) 1.09 mg/L.

Keywords: Antioxidant, Permeate, Polyphenols, Green tea .



INTRODUCTION

Antioxidants are necessary dietary components and there has recently been a lot of interest in their use. Vitamins (vitamin E, C, and -carotene) and plant polyphenols are natural antioxidants found in plants and food additives. Synthetic antioxidants such as butylated hydroxytoluene (BHT) are less effective than natural antioxidant owing to their abilities to preserve food from free radicals, natural antioxidants can be found in nutraceuticals and functional food plants and reactive oxygen species (ROS) damage and decrease in the risk of chronic disease. (Finley *et al.*2011).

The by-product of milk ultrafiltration is a large amount of permeate.. It comprises lactose as the major constituent (65% to 85%), (Menrad *et al.*, 2000) As a result, permeate can be used as a rich nutrient fluid. Milk permeate is a good source of important vitamins and minerals for human health. Permeate is similar to a challenge for dairy businesses in terms of disposal as a pollutant source, but much emphasis has been paid to employing permeate in the manufacturing of various beneficial products. (ElNawawy *et al.*, 2009). Calcium, sodium, magnesium, and potassium are minerals found in milk permeate that have electrolytic characteristics. (Fontes, Alves, Fontes, & Minim, 2015). Milk permeate contains these electrolytes could be used in beverages (Debon and Elane, 2012). Milk permeate is high in minerals that aren't found in alternative foods, and it enhances a food's total nutritional value. (Fitzpatrick *et al.*, 2001). Permeate has economic implications so it can be used as alternative for skim milk as a partial or whole replacement,

Green tea is one of the most popular beverages in the world.. (Reygaert, 2018). Catechins are the most common

type of antioxidant as polyphenols found in green tea. Green tea drinking is linked to health advantages in conditions such as atherosclerosis and cancer. Green tea has been proven to have anticarcinogenic, anti-inflammatory, antibacterial, and antioxidant qualities, making it useful in the treatment of cardiovascular disease (CVD), diabetes and obesity, as well as neurologic and dental health. (Crew *et al.* 2015, Li *et al.* 2014, Shirakami *et al.*2016, Subramani and Natesh, 2013).

People all over the world are drinking beverages made from natural fruit juices on a regular basis. Fruit juices are high in nutrients, including a variety of vitamins, minerals, and proteins and a diverse source of protective antioxidants, and they can also provide equivalent energy. Banana fruits (*Musa acuminata*) are especially relevant owing to their aroma and flavor and are classed as one of the most widely consumed fruits on the planet (Sulaiman *et al.* 2011). Carbohydrate, protein, vitamins A and C, sodium, calcium, magnesium, and potassium are all found in bananas. (Fabiano *et al.* 2006). The value of antioxidants of bananas characterized in ORAC units is 1,037 mmol TE, which is similar to orange juice and kiwi fruit. (U.S. Department of Agriculture.2007).

Pomegranate (*Punica granatum L.*) is reported to have major beneficial for good health with antiviral, antimicrobial, antioxidant, anticancer and anti-mutagenic properties. (Negi *et al.* 2003).

The fresh juice is 85.4 percent water and includes considerable levels of anthocyanins, phenolics, ascorbic acid, total soluble solids (TSS), total sugars, reducing sugars and proteins, as well as being strong source of antioxidants. Many additional antioxidants, such as vitamin C, vitamin E, coenzyme Q-10, and alpha-lipoic acid, are less potent than these antioxidants. (Aviram *et al.* 2002). Pomegranate juice contains more antioxidants than green tea and red wine

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according to research. (Gil *et al.* 2000). Many phenolic chemicals are found in pomegranate fruit such as anthocyanins, other complex flavonoids, and hydrolysable tannins are among the flavonoids (punicalagin, gallic and ellagic acid), which are substances have a lot of antioxidants that could be beneficial to health. Hydrolysable tannins account for around 92 percent of pomegranate antioxidant activity. Punicalagin, ellagic acid and gallic acid are the polyphenols found in pomegranate.

Citrus fruits (lemons and oranges) have been an important part of a balanced and nutritious diet and it is well established that certain of the nutrients included in citrus promote health and give protection from chronic illness (Adibelli *et al.*, 2009) Citrus fruits are the most antioxidant-rich of all the fruits, may help to avoid cancer, heart disease, cataracts, macular degeneration, and infection. Citrus liquids, particularly lemon juice, have the highest flavonoid content (800- 1500 mg l⁻¹), especially flavone and flavone glycosides. (González *et al.*, 2008). Hesperidin and eriocitrin (flavones) (flavonoids) are the most numerous among the flavones (such as 90 percent in lemon). Moreover, on the hand minor flavonoids identified within lemon juice are iso/limocitrol 3-β-glucoside and limocitrin 3-β-glucoside (Chornomaz *et al.*, 2013). Oranges consider a significant source of antioxidants (especially vitamin C and polyphenolic compounds) in the diet, (I. Klimczak *et al.*, 2007). Vitamin C content in orange juice is about 30 mg per 100 m, (V. Kabasakalis *et al.* 2000), This means that one glass (250 mL) contains 60–70% of the daily recommended amount. In addition to vitamin C, polyphenolic chemicals, the most common of which are hydroxycinnamic acid and flavanones, play an important role in the antioxidant effects of orange juice. Anti-inflammatory, antihypertensive, diuretic, analgesic, and hypolipidemic effects have been demonstrated in these substances. (I. Klimczak *et al.*, 2007). Strawberries (*Fragaria xananassa*) are high in phenols and especially a class of phenols called anthocyanins. Strawberries contain anthocyanins, which give the fruit its red colour and act as a powerful antioxidant, protecting the body's cells from free radical damage. Strawberry is a good source of minerals and vitamins such as vitamin C and B₃, carbohydrates, organic acid, flavonoids, pectin and mineral compounds (Cordenunsi *et al.*, 2002 and Li, 2008). Strawberries are a high source of anthocyanins in human nutrition, with pelargonidin-3-O-glucoside accounting for 77–90% of total anthocyanins in strawberries. (Lopes *et al.*, 2007). This study aimed to evaluate antioxidant, ascorbic acid and minerals content of different fruits (banana, pomegranate, orange and strawberry) permeate beverage enriched with green tea extract.

MATERIALS AND METHODS

Permeate was obtained from dairy factory in Dakahlia governorate. Green tea, banana, pomegranate, orange, strawberry, lemon, sugar and salt were obtained from local market Gharbia governorate.

Preparation of fruit juice and green tea extract

Fruits (banana, pomegranate, orange, strawberry and lemon) were washed, muslin cloth was used to filter the mixture after it was combined in a mixer grinder. Green tea (15 g) was placed in a beaker with 40 ml boiled water (90°C) and covered for 10 minutes with aluminium foil. The green

tea water extract was filtered before being chilled and kept at 5°C in the refrigerator. (Atallah and Gemiel, 2020).

Preparation of permeate, fruit juice and green tea extract beverages

Permeate, fruit juice and green tea extract beverages were prepared in the method shown. in Table1. The beverages were examined for physicochemical, minerals, antioxidant and vitamin C.

Table 1. Formulas of permeate, fruit juice and green tea extract beverages

Component	B1(%)	B2(%)	B3(%)	B4(%)
Permeate	40	40	40	40
Banana juice	40	-	-	-
Pomegranate juice	-	40	-	-
Orange juice	-	-	40	-
Strawberry juice	-	-	-	40
Green tea extract	10	10	10	10
Sugar	4	4	4	4
Water	3.5	3.5	3.5	3.5
Lemon juice	2	2	2	2
Nacl	0.5	0.5	0.5	0.5

B1= Permeate banana green tea extract beverage

B2=Permeate pomegranate green tea extract beverage

B3=Permeate orange green tea extract beverage

B4=Permeate strawberry green tea extract beverage

Phenolic acids profile

The sample (1 g) was put into 20 ml 2M NaOH in a quick-fit conical flask, flushed with N₂, and the stopper replaced. At room temperature. The samples were shackled for four hours. With 6 M HCl, the pH was adjusted to 2. The samples were centrifuged at 5000 rpm for 10 minutes, and the supernatant was collected. Phenolic compounds were extracted twice using a 1:1 mixture of 50 mL ethyl ether and ethyl acetate. The samples were redissolved in 2ml methanol after the organic phase was separated and evaporated at 45°C.

HPLC analysis was performed using an Agilent Technologies 1100 series liquid chromatograph with an auto sampler and a diode-array detector. A Eclipse XDB-C18 (150 X 4.6 m; 5 m) analytical column with a C18 guard column was used (Phenomenex, Torrance, CA). Acetonitrile (solvent A) and 2% acetic acid in water (v/v) made up the mobile phase (solvent B). For a total of 70 minutes, the flow rate was maintained at 0.8 ml/min and the gradient programme was as follows: 100% B to 85% B in 30 minutes, 85% B to 50% B in 20 minutes, 50% B to 0% B in 5 minutes, and 0% B to 100% B in 5 minutes. The injection volume was 50 l, and At 280 and 320 nm, the peaks of benzoic acid and cinnamic acid derivatives were examined simultaneously. All samples were filtered using a 0.45 m Acrodisc syringe filter prior to injection (Gelman Laboratory, MI). The peaks were identified using congruent retention durations and UV spectra, which were then compared to the standards.

Determination of total soluble solids, pH and minerals

The beverages were characterized in relation to total soluble solids and pH based on the methods mentioned by AOAC (2000). Minerals content (Ca, K, Mg and Na) were determined by instrument Inductively Coupled Plasma (ICP) model Optima 7000 DV according to the method of James (1995).

Radical DPPH scavenging activity

Free radical scavenging capacity was determined by the stable 1,1-Diphenyl-2-picryl-hydrazyl (DPPH). For

DPPH•, the final concentration was 50 µM and the reaction volume was 3.0 mL. The absorbance at 517 nm (A) was measured against a blank of pure methanol at 60 min. Percent inhibition of the DPPH free radical was calculated by the following equation:

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

A calibration curve was created to determine the antioxidant activity, with ascorbic acid, and expressed as mg of Trolox equivalent (TE) per gram of sample.

Determination of vitamin C

The method used 2, 6-dichlorophenol-indophenol titration to measure ascorbic acid concentration described in AOAC (2000). The Ultra-Turrax homogenizer was used to homogenise one gramme of material in a 20 ml cold solution of 3 percent (w/v) oxalic acid and 8 percent glacial acetic acid (v/v) in water until uniform consistency was attained for vitamin C analysis. The homogenates were centrifuged for 10 minutes at 10,000 rpm at 4°C. The supernatant was collected and promptly tested for vitamin C using 2,6 dichlorophenol dye.

Sensory evaluation

Acceptance was assessed according to the attributes color, flavor, aroma and total acceptability using 10 experienced panelists the according to Lawless & Heymann (2010). At each evaluation, the evaluators were instructed to rinse the mouth with water to remove possible residues inside the mouth.

Statistical analysis

The results were determined using analysis of variance significant differences between the results (ANOVA). Three duplicate parameters were subjected to a one-way analysis of variance at P<0.05.

RESULTS AND DISCUSSION

Phenolic compounds of green tea by HPLC

Table (2) shows phenolic compounds for green tea by HPLC. Green tea is a good source of phenolic compounds. The results indicated that the highest compound of phenolic compounds of green tea is Gallic acid (5724.21 µg/g) followed by Catechine, rutin, Cinnamic acid and Caffeic acid (3273.30, 239.823, 223.65 and 150.44µg/g) and the lowest value of phenolic compound is Chlorogenic acid (2.50 µg/g). Caffeine (not phenolic compound) value was 5545.18 µg/g. Gallic acid's cytotoxic and antitumor effects are mediated through changes in the antioxidant/pro-oxidant balance. In some situations, the chemical can reduce ROS-induced carcinogenesis by enhancing the activity of superoxide dismutase (SOD), catalase, and other antioxidant enzymes (CAT) ,glutathione reductase (GR) and glutathione peroxidase (GPx) and/or by reducing the lipid peroxidation and ROS production. Gallic acid, by activating the caspases pathway and creating reactive oxygen species (ROS) , can also cause cell cycle arrest, autophagy, and death.Catechins have been demonstrated to have a wide range of antibacterial effects, both in terms of organisms and processes. Green tea consumption has been demonstrated to transfer these chemicals and/or their metabolites throughout the body, allowing not only for the treatment but also for the prevention of infections. (Reygaert, 2018).Polyphenols are thought to have a variety of health benefits, including the prevention of inflammation, cancer, neurological illnesses, and diabetes, due to their antioxidant capabilities. (Cizmarova *et al.*,2020)

Table 2. Phenolic compounds of green tea by HPLC

Compound	Result (conc µg/g)
Gallic acid	5724.21
Protocatechuic acid	101.77
<i>p</i> -hydroxybenzoic acid	45.59
Catechine	3273.30
Chlorogenic acid	2.50
Caffeic acid	150.44
Ferulic acid	18.98
Sinapic acid	3.948
<i>p</i> -coumaric acid	8.84
Rutin	239.82
Cinnamic acid	223.65
quercetin	24.98
Kaempferol	8.53
Caffeine (not phenolic compound)	5545.184

Physicochemical of permeate, fruit juice and green tea extract beverages

Table (3) shows physicochemical of permeate fruit juice and green tea extract beverages which indicate that TSS of B₁ (Permeate banana green tea extract beverage) was the highest value (15.5) compared with B₂ (Permeate pomegranate green tea extract beverage), B₃ (Permeate orange green tea extract beverage) and the lowest value is for B₄ (Permeate strawberry green tea extract beverage) that is may be the cause of high of TSS of banana juice compared with pomegranate, orange and strawberry. The values of pH were (5.01), (4.02), (4.65) and (4.07) for B₁,B₂,B₃ and B₄, respectively.

Table 3. Physicochemical of permeate, fruit juice and green tea extract beverages

Parameters	B ₁	B ₂	B ₃	B ₄
TSS	15.5 ^a	14.5 ^b	12 ^c	11.5 ^d
pH	5.01 ^a	4.02 ^c	4.65 ^b	4.07 ^c

^{a-d} The different superscript letter have a significant difference in the same raw. (Duncan's test P<0.05)

B₁= Permeate banana green tea extract beverage

B₂=Permeate pomegranate green tea extract beverage

B₃=Permeate orange green tea extract beverage

B₄=Permeate strawberry green tea extract beverage

ANOVA studies of TSS values in beverages had shown significances (P<0.05) between all treatments. There is no significant between pH values between B₂ and B₄ but there are significant differences in pH between B₁ and B₃.These results are in agreement with (Atallah and Gemiel, 2020) who studied carbonated whey beverages fortified with fruit juice and some herbs extract.

Mineral content of permeate, fruit juice and green tea extract beverages

Table (4) shows mineral content of permeate, fruit juice and green tea extract beverages. The highest value of calcium content is 11.60 mg/L for B₄ (Permeate strawberry green tea extract beverage) while the lowest value is 7.27 for B₁ (Permeate banana green tea extract beverage). There are significant differences between all treatments. The values of K ranged between 37.52 and 53.48 for B₃ and B₂, respectively. Mg content ranged between 1.61 and 3.44 for B₂ and B₄, respectively. The highest value of Na is for B₂ and the lowest value of Na is for B₁. There are significant differences between all treatments in all minerals. Rizk (2016) showed that negligible changes in Mg, Na, and K for a lemon beverage while Guava beverage showed an increase in Ca content after mixing permeate with Guava fruit.

Table 4. Mineral content of permeate fruit juice and green tea extract beverages

Minerals (mg/L)	B ₁	B ₂	B ₃	B ₄
Ca	7.27 ^d	7.62 ^c	9.22 ^b	11.60 ^a
K	48.25 ^b	53.48 ^a	37.52 ^d	42.89 ^c
Mg	3.28 ^b	1.61 ^d	2.18 ^c	3.44 ^a
Na	104.70 ^d	162.70 ^a	138.50 ^b	130.70 ^c

^{a-d} The different superscript letter have a significant difference in the same raw. (Duncan's test P<0.05)

(Duncan's test P<0.05).

B₁= Permeate banana green tea extract beverage

B₂=Permeate pomegranate green tea extract beverage

B₃=Permeate orange green tea extract beverage

B₄=Permeate strawberry green tea extract beverage

Antioxidant activity of permeate fruit juice and green tea extract beverages

The antioxidant activity of permeate fruit juice and green tea extract beverages are described in Fig1. The highest percentage (P<0.05) was that corresponded to treatment B₄ (Permeate strawberry green tea extract beverage) (872.47 µg TE/g) followed by B₂ (Permeate pomegranate green tea extract beverage) (724.21 µg TE/g), B₃ (Permeate orange green tea extract beverage) (568.74 µg TE/g) and the lowest value was (93.79 µg TE/g) for B₁ (Permeate banana green tea extract beverage).

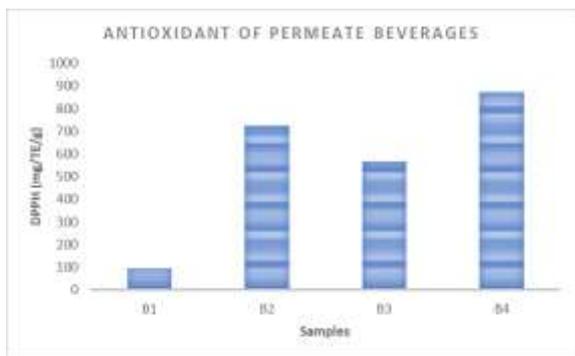


Fig.1. Antioxidant activity of permeate fruit juice and green tea extract beverages

B₁= Permeate banana green tea extract beverage

B₂=Permeate pomegranate green tea extract beverage

B₃=Permeate orange green tea extract beverage

B₄=Permeate strawberry green tea extract beverage

Permeate fruit juice and green tea extract beverages is being used as an ascorbic acid source which is vital vitamin in the human diet (Fig. 2).

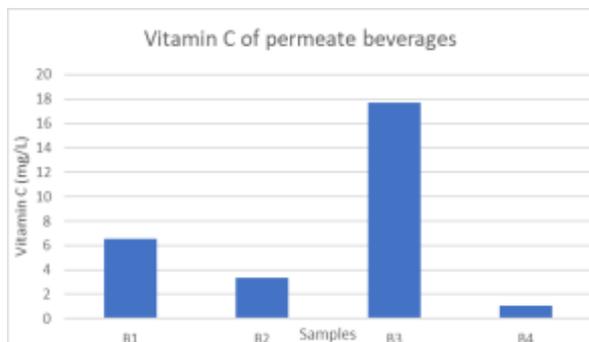


Fig.2. Ascorbic acid (Vitamin C) of permeate fruit juice and green tea extract beverages

B₁= Permeate banana green tea extract beverage

B₂=Permeate pomegranate green tea extract beverage

B₃=Permeate orange green tea extract beverage

B₄=Permeate strawberry green tea extract beverage

Vitamin C (ascorbic acid) levels were significant differences (P<0.05) between all samples. The highest value of ascorbic acid was for B₃ (Permeate orange green tea extract beverage) 17.20 mg/L followed by B₁ (Permeate banana green tea extract beverage) 6.53 mg/L, B₂ (Permeate pomegranate green tea extract beverage) 3.32 mg/L and the lowest value was for B₄ (Permeate strawberry green tea extract beverage) 1.09 mg/L. These results are in agreement with Atallah and Gemiel (2020) who found that the biggest ascorbic acid values in carbonated whey beverages containing lemon.

Sensory evaluation of permeate, fruit juice and green tea extract beverages

Sensory evaluation of beverages was evaluated as shown in Table (5). There are significant differences for all samples (P<0.05). The highest value for overall acceptability for B₄ (Permeate strawberry green tea extract beverage). It may be that strawberry improved color and taste compared with B₁ (Permeate banana green tea extract beverage), B₂ (Permeate pomegranate green tea extract beverage) and B₃ (Permeate orange green tea extract beverage).

Table 5. Sensory evaluation of permeate, fruit juice and green tea extract beverages

Parameter	B ₁	B ₂	B ₃	B ₄
Color	6.21	7.20	7.95	8.20
Flavor	7.11	7.01	7.50	8.11
Aroma	7.50	7.02	7.54	8.13
Overall impression	6.94 ^d	7.07 ^c	7.66 ^b	8.11 ^a

^{a-d} The different superscript letter have a significant difference in the same raw (Duncan's test P<0.05)

B₁= Permeate banana green tea extract beverage

B₂=Permeate pomegranate green tea extract beverage

B₃=Permeate orange green tea extract beverage

B₄=Permeate strawberry green tea extract beverage

CONCLUSION

Permeate with banana, pomegranate, orange and strawberry based on green tea extract beverages were analyzed for antioxidant, minerals and ascorbic acid content of these beverages. Gallic acid has the highest polyphenol content in green tea. Green tea is anticarcinogenic, anti-inflammatory, antibacterial, and antioxidant making it useful in the treatment of cardiovascular disease. The highest content of antioxidant is for permeate with strawberry juice based on green tea extract. The highest value of ascorbic acid is for permeate with orange juice based on green tea extract. Gallic acid has the highest polyphenol content in green tea.

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محتوى مضادات الأكسدة والمعادن لمشروبات مترشح اللبن مع عصير الفواكه ومستخلص الشاي الأخضر

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تم تحضير مشروب راشح اللبن مع عصائر الموز والبرنقال والفرولة مع مستخلص الشاي الأخضر وتم تقدير الخواص الحسية والمواد الصلبة والأمس الهيدروجيني ومضادات الأكسدة وبعض الأملاح المعدنية وحضض الاسكوربيك لهذه المشروبات. ولقد تم تقدير المركبات الفينولية للشاي الأخضر بواسطة HPLC. وأوضحت النتائج أن حمض الجاليك (5724.21 ميكروجرام/جرام) على التوالي. وكانت أعلى قيمة للمواد الفينولية يتبعها Catechine ، rutin ، Caffeic acid ، 239.823 ، 3273.30 ، 223.65 ، 150.44 ميكروجرام/جرام) على التوالي. وكانت أقل قيمة للمواد الفينولية لـ Chlorogenic acid بقيمة (2.50 ميكروجرام/جرام). ولقد كانت هناك فروق معنوية عند مستوى معنوية (P<0.05) في المعادن لجميع المعاملات. وكانت أعلى قيمة لمضادات الأكسدة (872.47 ميكروجرام TE/جرام) لمشروب B₄ (مشروب راشح اللبن مع عصير الفروالة ومستخلص الشاي الأخضر) يتبعها عينة B₂ (مشروب راشح اللبن مع عصير الرمان ومستخلص الشاي الأخضر) بقيمة (724.21 ميكروجرام TE/جرام) ويليه مشروب B₃ (مشروب راشح اللبن مع عصير البرتقال ومستخلص الشاي الأخضر) بقيمة (568.74 ميكروجرام TE/جرام) وكانت أقل قيمة لمشروب B₁ (مشروب راشح اللبن مع عصير الموز ومستخلص الشاي الأخضر) بقيمة (93.79 ميكروجرام TE/جرام). وكانت أعلى قيمة لحمض الاسكوربيك للمشروب B₃ (مشروب راشح اللبن مع عصير البرتقال ومستخلص الشاي الأخضر) بقيمة 17.20 مجم/لتر وكانت أقل قيمة للعينة B₄ (مشروب راشح اللبن مع عصير الفروالة ومستخلص الشاي الأخضر) بقيمة 1.09 مجم/لتر.

الكلمات الدالة: مضادات الأكسدة - راشح اللبن - الفينولات العديدة - الشاي الأخضر