

Functional properties and GC-MS Analysis of Phenolic Compounds in Developed Carbonated Whey Beverage with Pomegranate Peel Extract and Guava Leaves Extract

Fathimath Naurin K.A.^{1*}, Marx Nirmal R.², Ayyavoo Preamnath Manoharan² and Karthikeyan N.³

¹College of Food and Dairy Technology,

Tamil Nadu Veterinary and Animal Sciences University, Koduveli, Chennai (Tamil Nadu), India.

²Department of Food Process Engineering, College of Food and Dairy Technology,

Tamil Nadu Veterinary and Animal Sciences University, Koduveli, Chennai (Tamil Nadu), India.

³Department of Dairy Microbiology, College of Food and Dairy Technology,

Tamil Nadu Veterinary and Animal Sciences University, Koduveli (Tamil Nadu), India.

(Corresponding author: Fathimath Naurin K.A. *)

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ABSTRACT: In an attempt to develop a functional carbonated whey beverage, lactose hydrolyzed whey was mixed with sugar (10%), pomegranate peel extract (3%) and guava leaves extract (3%) and it was carbonated. Functional properties like ascorbic acid content, total phenolic content and antioxidant activity during storage at 4° showed that the developed carbonated whey beverage is antioxidant rich and the antioxidant activity was retaining during the storage period at 4°. The developed carbonated whey beverage showed an antioxidant activity (75.2%) DPPH radical scavenging activity, total phenolic content (125.12 mg GAE/100mL) and ascorbic acid content (13.05 mg/100g) in it. A gradual decrease in functional properties was observed throughout the storage period. GC-MS analysis of beverage added with extracts showed the presence of phenolic compounds like gallic acid, ferulic acid, ellagic acid, punicalagin and Peduncalagin I. Presence of these compounds indicates the potency of using pomegranate peel extract and guava leaves extract as a source of powerful antioxidants. The present study implements the proper utilization of by-products.

Keywords: Hydrolyzed whey, guava leaves extract, antioxidant activity, Total phenolic content, ascorbic acid, GC-MS. Pomegranate peel.

INTRODUCTION

Recently, antioxidants have attracted considerable attention in relation to radicals and oxidative stress, cancer prophylaxis and therapy and longevity. The recommendations based on epidemiological studies are fruits, vegetables and less processed staple foods ensure the best protection against the development of diseases caused by oxidative stress such as cancer, coronary heart disease, obesity, type 2 diabetes, hypertension and cataract (Halvorsen *et al.*, 2002). The explanation consists in the beneficial health effect, due to antioxidants present in fruit and vegetables (Halvorsen *et al.*, 2006).

the substantial quantities of phenolic compounds such as ellagitannins, ellagic acids and gallic acids had been attributed to the antioxidant potential of pomegranate peel. Pomegranate peel is recognized as a potential source of antioxidants for the stabilization of food systems (Yasoubi *et al.* 2007; Ibrahim, 2010). Pomegranate fruit peel has antioxidant and antimutagenic properties. It shows the antioxidant activity due to the presence of polyphenols such as ellagitannins, ellagic acid and gallic acid. The methanolic extract of fruit peel contain high antioxidant activity but weak antimutagenic activity. The water

extract shows less antioxidant activity and high antimutagenic activity (Negi *et al.*, 2003). Walid *et al.* (2012) reported that pomegranate (*Punica granatum* L.) is a nutrient dense food which is rich in beneficial phytochemicals.

Guava leaves are cheaply available and their extract shows high antioxidant activity and free radical-scavenging ability (Chen and Yen 2007). Highest antioxidant activity found in young leaves extract. Hot water is the best medium of extraction of active components and ultrasonication is the best method for the extraction (Nantitanon *et al.*, 2010). The guava leaves extract has been successfully incorporated in many foods as a source of antioxidant.

Around nine compounds having some biological activities were identified in guava leaves extract using GC-MS (Devi *et al.*, 2018). Afzal *et al.* (2019) studied the characteristic metabolic compounds in guava leaves extract by GC-MS and HPLC especially the phenolic compounds present in the extract.

Functional beverages are mostly accepted by all the age group and hence this study is anticipated to develop a carbonated whey beverage with utilization of pomegranate peel and guava leaves extract as a source of natural antioxidants and phenolic compounds.

MATERIAL AND METHODS

Materials. Whey (paneer whey) was provided by the Incubation centre of College of Food and Dairy Technology, Chennai, Tamil Nadu. The lactase enzyme (Maxilact® L 5000) was purchased from DSM Food specialties, Denmark. Good grade sugar and pomegranate peel was purchased from local market-Redhills, Chennai. Fresh Guava leaves were collected from guava trees of college compound, CFDT-Alamathi.

Methods

Preparation of pomegranate peel and guava leaves extract. The washed and cleaned pomegranate peel and guava leaves were cut in to small pieces of about 0.5 cm² and dried at 40° for 12 hours in the solar dryer and it was powdered. 5 g pomegranate peel powder was added to 300 ml of boiling water and kept for 5 minutes. Then the mixture is cooled to 37° and left in dark at room temperature for 1hour. Then the extract is filtered through whatman No.1 filter paper, resulting extract is centrifuged at 3500 rpm for 10 minutes. 20 grams of guava leaves powder was added to 500 ml of boiling water and kept for 5 minutes. Then the extract is filtered through whatman No. 2 filter paper. (Chen and Yen 2007; Jalal *et al.*, 2018).

Preparation of Carbonated whey beverage. The filtered and cream separated fresh paneer whey was pasteurized at 80 °C for 10 minutes and cooled to 37 °C. Using 1N KOH the pH of whey was adjusted to 6.6 for the lactose hydrolysis. Enzyme (Maxilact® L 5000 Lactase enzyme) was added at 0.3ml/litre and then incubated at 40 °C for 3 hours in the incubator (Mabrouk and Gemiel 2020). The enzyme was inactivated by heating at 80° C for 5 minutes After the incubation. The other ingredients like Sugar (10%), Pomegranate peel extract (3%) and Guava leaves extract (3%) were added to the hydrolyzed and cooled (37 °C) whey. Finally the whey was pasteurized at 80° C for 10 minutes followed by immediate cooling to 5° C. The beverage was carbonated at 15 psi pressure, filled and crown corking in glass bottle and kept for refrigerated storage (4°).

Functional properties of carbonated whey beverage.

The ascorbic acid content in the samples were assessed by the method of visual titration with the use of freshly prepared 2, 6-dichlorophenolindophenol dye solution (Ranganna, 2005). The total phenolic contained in the developed carbonated whey beverage was determined according to method adopted by Singleton *et al.* (1965). The total antioxidant activity of carbonated whey beverage was determined as per the DPPH method brought up by Brand-Williams *et al.* (1995).

Phenolic compounds analysis by Gas Chromatography-Mass spectrometry.

Gas chromatography (Agilent: GC: (G3440A) 7890A. MS MS:7000 Triple Quad GCMS) equipped with mass spectrometry detector was used for analysis of phenolic compounds of developed beverage. The oven temperature was programmed from 60° (isothermal for 2 minutes), with an increase of 25°/min, to 310° isothermal for 11.0 min. A split injection was used for sample introduction and the split ratio was set to 1:10.

The helium carrier gas was set to 1.8mL/minute flow rate (constant flow mode). The mass spectrometer operating in electron ionization (EI) mode with TSS-2000 software was used for analysis. electron impact mode was at 70Ev. The ionization temperature was at 280°. Electronic ionization from 35to 700m/z (Toure *et al.*, 2011; Fernandes *et al.*, 2014).

Statistical analysis. One way analysis of variance (ANOVA) used for performing statistical analysis. Statistical analysis was conducted with IBM SPSS® 20.0 for Windows® software program.

RESULTS AND DISCUSSION

Functional properties of carbonated whey beverage.

The mean of ascorbic acid content in control in the 0th, 14th, 28th, 42nd and 56th day of storage at 4° is 2.14, 2.04, 1.95, 1.68 and 1.42 mg/100g respectively as given in table 1. The mean of ascorbic acid content in carbonated whey beverage in the 0th,14th, 28th,42nd and 56th day of storage at 4v is 13.05, 12.39, 12.03, 11.53 and 11.04mg/100g respectively. A significant (P≤0.01) decrease was observed in ascorbic acid content of control and carbonated whey beverage during storage period. The decrease in ascorbic acid content is due to the oxidation of ascorbic acid to dehydroascorbic acid in presence of oxygen (Sharma *et al.*, 2019). The results are correlated with Mokhtar and Ibrahim (2020). The decrease in ascorbic acid content during storage period was observed by Mokhtar and Ibrahim (2020) in their guava nectar.

The mean of total phenolic content in control in the 0th, 14th, 28th, 42nd and 56th day of storage at 4° were 9.54, 9.13, 8.72, 8.28 and 7.84 mg GAE/100mL respectively as given in table 2. The mean of total phenolic content in carbonated whey beverage in the 0th, 14th, 28th, 42nd and 56th day of storage at 4° were 125.12, 116.27, 110.43, 108.90 and 105.38mg GAE/100mL respectively. The total phenolic content of control and carbonated whey beverage decreased from 9.54 to 7.84 mg GAE/100mL and 125.12 to 105.38 mgGAE/100mL respectively after 56 days of storage at 4°. The decrease in TPC is due to the condensation of polyphenols in to brown pigments (Muzaffar *et al.*, 2017). The same trend was also observed in guava nectar by Mokhtar and Ibrahim (2020).

The mean of antioxidant activity in control in the 0th, 14th, 28th, 42nd and 56th day of storage at 4° were 14.5, 13.5, 12.4, 11.8 and 11.2% radical scavenging activity respectively as given in table 3. The mean of antioxidant activity in carbonated whey beverage in the 0th, 14th, 28th, 42nd and 56th day of storage at 4° is 75.2, 71.8, 68.3, 62.9 and 57.5% radical scavenging activity respectively. The antioxidant activity of control and product are significantly (P ≤ 0.01) decreased after 56 days of storage at 4°. The decrease in antioxidant activity was observed due to polyphenols condensation into brown pigments (Muzaffar *et al.*, 2017). The results are correlated with Mokhtar and Ibrahim (2020) found that there is an increase in antioxidant activity with increase in concentration of extracts. Chen and Yen (2007) also showed similar result.

Table 1: Ascorbic acid content (Mean±SE) @ of carbonated whey beverage during storage at 4°C.

| Storage Days | Ascorbic acid content (mg/100g) | | t value |
|------------------|---------------------------------|--------------------------|----------|
| | Control | Carbonated whey beverage | |
| 0 th | 2.14±0.09 ^d | 13.05±0.16 ^e | 58.200** |
| 14 th | 2.04±0.01 ^c | 12.39±0.15 ^d | 66.187** |
| 28 th | 1.95±0.02 ^c | 12.03±0.15 ^c | 60.848** |
| 42 nd | 1.68±0.0 ^b | 11.53±0.09 ^b | 78.422** |
| 56 th | 1.42±0.02 ^a | 11.04±0.13 ^a | 43.340** |
| F value | 40.489** | 266.214** | - |

@ Average 6 trials; ** Highly significant (P≤0.01) difference. Means bearing various superscripts in the same column differs highly significantly

Table 2: Total phenolic content (Mean±SE) @ of carbonated whey beverage during storage at 4°C.

| Storage Days | Type of beverage | | t value |
|------------------|------------------------|---|----------|
| | Control (mg GAE/100mL) | Carbonated whey beverage (mg GAE/100mL) | |
| 0 th | 9.54±0.05 ^c | 125.12±1.27 ^e | 90.273** |
| 14 th | 9.13±0.06 ^d | 116.27±0.98 ^d | 91.923** |
| 28 th | 8.72±0.08 ^c | 110.43±0.68 ^c | 96.671** |
| 42 nd | 8.28±0.10 ^b | 108.90±1.08 ^b | 48.499** |
| 56 th | 7.84±0.10 ^a | 105.38±0.81 ^a | 46.933** |
| F value | 61.846** | 61.846** | - |

@ Average 6 trials; ** Highly significant (P≤0.01) difference. Means bearing various superscripts in the same column differs highly significantly

Table 3: Antioxidant activity (Mean±SE) @ of carbonated whey beverage during storage at 4°C.

| Storage Days | Type of beverage | | t value |
|------------------|---|---|----------|
| | Control (%DPPH radical scavenging activity) | Product (%DPPH radical scavenging activity) | |
| 0 th | 14.5±0.18 ^d | 75.2±0.96 ^e | 61.553** |
| 14 th | 13.5±0.18 ^c | 71.8±0.85 ^d | 66.965** |
| 28 th | 12.4±0.25 ^b | 68.3±0.79 ^c | 67.140** |
| 42 nd | 11.8±0.13 ^a | 62.9±0.75 ^b | 66.690** |
| 56 th | 11.2±0.25 ^a | 57.5±0.73 ^a | 59.515** |
| F value | 40.428** | 73.168** | - |

@ Average 6 trials; ** Highly significant (P≤0.01) difference. Means bearing various superscripts in the same column differs highly significantly

Phenolic compounds analysis by Gas Chromatography-Mass spectrometry. GC-MS analysis of the control and developed whey beverage is presented in Table 4 and their chromatograms are shown in Fig. 1 and 2.

Phenolic compounds like gallic acid, Ferulic acid, ellagic acid, gallic acid, gallo catechin, *p*-Coumaric acid, Punicalin, Peduncalagin I and 2,4,6-Cycloheptatrien-1-one,3,5-Bis-Trimethylsilyl compounds was found to be 10.88, 19.01, 9.84, 5.87, 0.61, 3.33, 8.94 and 7.67 % respectively in the sample. All the phenolic compounds were not detected in control. Phenolic compounds like gallic acid, Ferulic acid, ellagic acid, gallo catechin, *p*-

Coumaric acid, Punicalin, Peduncalagin I and 2,4,6-Cycloheptatrien-1-one,3,5-Bis-Trimethylsilyl compounds were detected in different peak area.

Product contain compounds with high peak area are ferulic acid, gallic acid and ellagic acid. Shaheena *et al.* (2019) detected phenolic compounds like gallic acid, ellagic acid in guava leaves extract. Al-hindi and Abd (2020) reported that the presence of phenolic compounds like ellagic acid, ferulic acid, *P*-coumaric acid and punicalagin was found in pomegranate peel extract. The phenolic compounds like ellagic acid and ferulic acid possess antioxidant, antimicrobial and anti-inflammatory actions.

Table 4: Phenolic compounds analysis by Gas Chromatography-Mass spectrometry.

| Compound name | Peak area (%) | |
|---|---------------|--------|
| | Control | Sample |
| Gallic acid | NIL | 10.88 |
| Ferulic acid | NIL | 19.01 |
| Ellagic acid | NIL | 9.84 |
| Gallo catechin | NIL | 5.87 |
| <i>p</i> -Coumaric acid | NIL | 0.61 |
| Punicalin | NIL | 3.33 |
| Peduncalagin I | NIL | 8.94 |
| 2,4,6-Cycloheptatrien-1-one,3,5-Bis-Trimethylsilyl- | NIL | 7.67 |

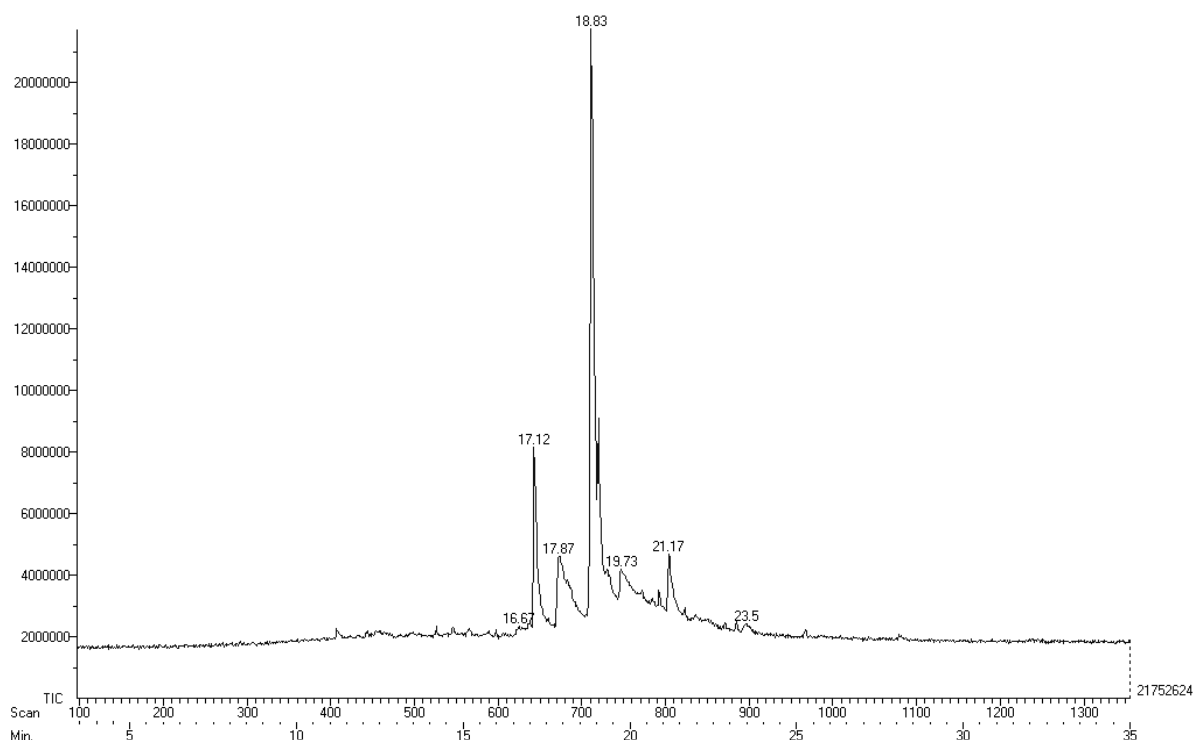


Fig. 1. GC-MS profile of control.

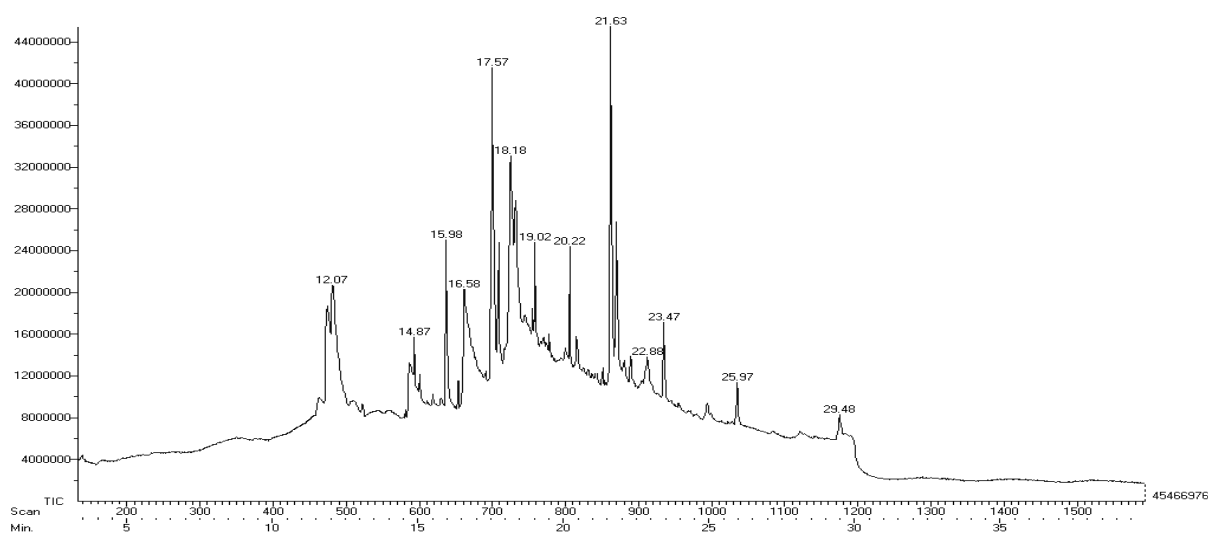


Fig. 2. GC-MS profile of whey beverage with pomegranate peel and guava leaves extract.

CONCLUSIONS

The results obtained showed that the addition of pomegranate peel and guava leaves extract enhanced the ascorbic acid, total phenolic content and antioxidant activity of the developed whey beverage. GC-MS analysis of the developed beverage shows the presence of phenolic compounds which possesses the antioxidant, antimicrobial and anti-inflammatory activity. It can be concluded that the developed beverage can be recommended as a functional carbonated beverage. Utilization of by-products as an antioxidant source are trending in research areas.

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Conflict of Interest. None.

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