***Original Research Article***

# Improving the antioxidant activity of a carbonated lemon soft drink


## ABSTRACT

Carbonated functional soft drink is a new concept that provides health benefits for the consumers. The aim of this study was to improve the antioxidant activity of a carbonated lemon soft drink, while preserving its characteristic flavour, by blending the lemon oil (L) with oregano (Or) essential oil. The effect of different thickeners, usually used in formulation of the soft drinks, on release of the volatile compounds from simple model solutions was evaluated, separately. Blending lemon oil with oregano essential oil (1.5 µL Or /100 µL) improved the odour acceptability and increased the DPPH scavenging ability of lemon oil by 40%. Studying the effect of different thickeners indicated that the xanthan, Arabic gum and pectin at levels 0.05, 6.0 and 0.01 g/100 mL water, respectively, showed the highest release of limonene and citral, the most contributors to lemon aroma, from the simple model solutions. This finding was mainly correlated to the interaction between the thickeners. In view of this result a lemon soft drink was formulated. Carbonation of the soft drink improved the sensory characteristics, decreased the pH value, increased the acidity and showed insignificant changes ($P > 0.05$) in total soluble solids and total sugars.



*Keywords: Lemon oil; oregano oil; carbonated soft drink; thickeners; sensory evaluation; antioxidant activity.*


## 1. INTRODUCTION

Carbonated soft drinks are highly favorite beverages by consumers all over the world, representing 25% of total beverage industry. Nowadays, there is a growing interest from industry to impart beverages functional properties that benefit the health of consumers [1]. The essential oil from fresh lemon peel, in addition to its characteristic citrus aroma and flavour [2], it has functional properties such as antibacterial, antifungal and anti-inflammatory [3]. It is known as a remedy for coughs [4,5].

Lemon based carbonated soft drink contains lemon oil with other ingredients such as citric acid, sodium citrate, emulsifier and preservatives [6]. Chemical preservatives are used to improve the microbiological and oxidative stability of soft drinks, however there is a growing interest to find alternative natural sources that could be used as food preservatives.

Several studies have been carried out on oregano essential oil (EO) and correlated its effective antioxidant ability and antimicrobial activity against a broad spectrum of microorganisms [7,8] to its high content of the phenolic compounds such as thymole and carvacrol. Oregano EO is commercially used as a food flavouring agent, as a fragrance compound in cosmetic industry [9] as well as a medicine for treating several diseases [10].

Gum Arabic, xanthan and pectin are the most used thickeners in soft drink industry [11]. In some times, they are used to improve the mouth feel, mask flavours and help in carbonation retention. Studying the effect of these compounds on the release of the

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volatile compounds from a soft drink can be useful for appropriate formulation of the end product [12-14].

Carbonation process is adding sufficient carbon dioxide gas ($CO_2$) to the beverage so that on serving product gives off $CO_2$ gas as fine bubbles and impart the beverage a distinctive taste and aroma. $CO_2$ have an effective action against the growth of microorganisms, thus prolongs the shelf-life of the beverage [15,16].

The main objective of the present study was to impart the carbonated lemon soft drink functional properties such as antioxidant. To achieve this aim the addition effect of oregano essential oil on the odour sensory quality and antioxidant activity of lemon essential oil was evaluated. Effect of the type/concentration of different thickeners, widely used in formulation of the soft drinks, on release of the volatiles from lemon essential oil-oregano essential oil (L-Or) blend was studied. The role of the viscosity on release of the volatile compounds from each thickened solution was evaluated. A lemon based soft drink was formulated in view of the obtained results. The carbonated soft drink was subjected to sensory and physicochemical analysis to assess the effect of carbon dioxide on its quality compared to a non carbonated soft drink.

## 2. MATERIALS AND METHODS

### 2.1 Materials

Cold pressed lemon oil (L) was purchased from El-Marwa Food Industries Company, Egypt. Oregano (Or) essential oil was hydrodistilled from clean air dried oregano plant purchased from Ferrous company, Giza, Egypt.

The authentic volatile compounds, standard n-alkanes ($C_8$-$C_{22}$), 2,2-diphenyl-1-picryhydrazyl ($DPPH^{\cdot}$) and 2,2-azinobis (2-ethyl-benzothiazoline-6-sulfonic acid) ($ABTS^{+}$), xanthan, pectin, Arabic gum, citric acid, sodium citrate, potassium persulfate and sucrose were purchased from Sigma Aldrich Chemical Co.(St. Louis, MO, USA) and Merck (Darmstadt, Germany). All other chemicals were of analytical grade and the solvents were purified and distilled before use. Mineral water, pH 7 (Dasani, Cocoa Cola Company, Egypt) was used throughout the study. Its mineral composition (mg/L) was as follows: calcium 18.5, magnesium 5.2, sodium 10.8, potassium 1.4, bicarbonate 72, sulphates 8.5, chlorides 8.4, silicate 8.0 and total dissolved salts (T.D.S) 110.

### 2.2 Evaluation of the odour quality and antioxidant activity of the lemon-oregano (L-Or) essential oil blends

Lemon oil (100 µL) was blended with 0.0 0.5, 1.0, 1.5 µL and 2 µL oregano EO. The odour quality of each blend was assisted by a well-trained panel consisting of 10 members drawn from Food Technology and Nutrition Institute, National Research Center, Cairo, Egypt. The panelists scored the odour acceptability of each sample on a category scale 0.0 (not perceptible) to 10.0 (strongly perceptible). Each sample was evaluated in triplicate.

The antioxidant activity (AOA) of lemon oil, oregano EO and their blends was assisted on the basis of the scavenging activity of the stable radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azinobis (2-ethyl-benzolhiaxoline-6-sulfonic acid) ($ABTS^{+}$) according to Yen & Chen [17] and Re et al. [18], respectively.

Inhibition of radical activity (%) = $[(A_0-A_1)/A_0] \times 100$

Where: $A_0$ is the absorbance of the control (solution without sample); $A_1$ is the absorbance of sample.

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### 2.3 Gas chromatography-mass spectrometry (GC-MS) analysis

The analysis of lemon EO and oregano EO and their blends was conducted by using a gas chromatography (Agilent 8890 GC system) coupled to a mass spectrometer (GC-MS) (Agilent 5977B GC/MSD) and equipped with DB5 fused silica capillary column (60 m x 0.32 mm i.d.x 0.25 µm film thickness) all other conditions were carried out according to the previous study [19].

### 2.4 Preparation of soft drink simple model systems

The blends of lemon essential oil (L-Or) that showed the best odour and antioxidant activity was used in preparation of the soft drink. Before formulation of the soft drink, the effect of different thickeners on release of the volatile compounds in the headspace of the simple model solution containing selected blend (L-Or) was studied, separately.

Simple model solution thickened with variable concentrations (0.05, 0.10, 0.20, 0.40 and 0.60% w/v) of xanthan [11], (2, 4, 6, 8 and 10% w/v) of Arabic gum and (0.05, 0.10, 0.20, 0.40 and 0.60% w/v) of pectin [20] were prepared, separately. The selected L-Or blend was added in concentration of 0.20% (v/v) to each thickened solution under stirring in closed system at 60 °C for 3 min. All model solutions were prepared at least 24 h before evaluation and stored at 4 °C [21]. The samples were brought to room temperature before isolation of the headspace volatiles.

### 2.5 Preparation of lemon soft drink

A mixture of xanthan, gum Arabic and pectin, at the concentration of each that showed optimal release of volatile compounds, was dissolved in 100 mL water. Sucrose (13.5 g) as a sweetener, sodium citrate (0.4 g) and the selected L-Or blend (0.2 mL) were added to the thickened solution and mixed thoroughly with magnetic stirring for 15 min at 60 °C. The pH of the stirred solution was adjusted at pH 5.0 by using citric acid (50 w /w) solution. The volatiles of the prepared soft drink were isolated by solid phase microextraction (SPME) and subjected to GC-MS analysis.

### 2.6 Isolation and analysis of the volatiles in headspace of the thickened model solutions

Optimization experiments revealed that the most appropriate conditions for the isolation of headspace volatiles by SPME were using the fiber Polydimethylsiloxane (PDMS) for 15 min at 45 °C. The soft drink (5 mL) was transferred into a 20 mL headspace vial sealed with a Teflon-Lind septa and screw cap and immersed in a water bath at 45 °C. The fiber was exposed to the sample headspace for 15 min to reach equilibrium and then was withdrawn into the needle holder and introduced immediately into the gas chromatography (GC) injection port. Analysis of the volatile compounds was performed by using a gas chromatography (Agilent 8890 GC system) coupled to a mass spectrometer (GC-MS) (Agilent 5977B GC/MSD). The injection was conducted in the splitless mode for 5 min at 250 °C. The GC was equipped with a fused silica capillary column DB5 (60 m x 0.32 mm i.d.x 0.25 µm film thickness). The oven temperature was hold initially at 50 ºC for 5 min, then programmed from 50 to 220 ºC at a rate of 4 ºC/ min, and from 220-280 ºC at a rate of 10 ºC/min. Helium was used as the carrier gas, at a flow rate of 1.1 mL/min. Mass spectra in the electron impact mode (EI) were obtained at 70 eV and scan m/z range from 39 to 400 amu. A series of alkanes ($C_8$ - $C_{22}$), as an external standard, was run under the same chromatographic conditions as the samples, to calculate the retention indices (kovats

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index) of the separated volatile compounds. The isolated peaks were identified by matching with data from the library of mass spectra (National Institute of Standard and Technology, NIST) and comparison with those of authentic compounds and published data. The amount of each individual separated compound was expressed as total ion chromatograms (TIC).

### 2.7 Viscosity measurement

Viscosity of the thickened model solutions and the formulated soft drink was carried out according to Darwish et al. [22] by using RheolabQC (C-PTD 180/AIR/QC, Anton Paar, German).

### 2.8 Preparation of the carbonated soft drink

Carbon dioxide gas ($CO_2$) from a carbonated maker (ISODA Industrial Company, Model: Drinkmate 410, Chain) was passed through a tube to a bottle made of a polymer material (polyethelene tetraphthalate, 01). The bottle was filled with the chilled soft drink (5 °C) up to a specific mark (800 mL) that allows the gas tubing to immerse into the soft drink. The carbonated soft drink was bottled in chilled bottles (0 °C) and immediately sealed by crown corking until further evaluation. Measurement of $CO_2$ concentration was conducted by using the titration method [15].

### 2.9 Physicochemical and sensory analysis of the carbonated and non carbonated soft drink

The carbonated and noncarbonated soft drink was analyzed for pH, acidity, Brix and total sugars [23]. The carbonated and non carbonated soft drinks were evaluated for sensory characteristics on the basis of aroma, taste, mouthfeel, colour and overall acceptability [24] on a 9- point hedonic scale (9 = like extremely to 1= dislike extremely). The test panel was conducted at room temperature by 20 panelists (Food Technology and Nutrition Institute). The panelists received the samples separately in colorless glass cups and provided with water to drink between evaluations.

### 2.10 Statistical analysis

Analysis was performed in triplicate for each sample for all the tests. Data were compared using one-way analysis of variance (ANOVA) by the statgraphics package (Statistical Graphics Corporation, 1993; Manugistics Inc., USA) and least significant differences (LSD) was performed to determine any significant difference amongst various treatments ($p < 0.05$). For interpreting the results, principal component analysis (PCA) was performed based on volatile compounds released from the simple model solutions. XLstat-Pro (Addinsoft) was applied according to Cozzolino et al. [25].

## 3. RESULTS AND DISCUSSION

### 3.1 Chemical composition of lemon and oregano EOs

The chemical composition of lemon oil (L) and oregano (Or) EO as well as their blends are shown in Table 1**.** The GC-MS analysis of lemon oil revealed the presence of 16 volatile compounds representing 97.49% of the total oil. D-Limonene was the major identified compound (71.50%) followed by sabinene (8.31%) and γ-terpinene (4.58%), β-pinene (2.02%), α-pinene (1.84%) and citral (neral and geranial, 1.84%). These results are consistent with those of Aguilar-Hernández et al. [26]. The importance of lemon oil in food and cosmetics industries is correlated to its content of

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limonene. Limonene and citral were described to have a lemon aroma [26]. Twenty-three volatile compounds were identified in Or EO, representing 98.99% of the total volatiles (Table 1). Thymol was the major compound (30.53%) followed by γ-terpinene (18.15%), α-terpineol (11.00%) and Linalool (7.32%) [27].

**Table 1. Volatile compounds identified in lemon oil (L), oregano EO (Or) and their blends**

| Peak No. | KI[a] | Volatile Compounds[b] | Area (%)[c] | | | | |
|---|---|---|---|---|---|---|---|
| | | | Lemon | Oregano | L+ 0.5 Or | L+ 1.0 Or | L+ 1.5 Or |
| 1 | 929 | α-Thujene | 0.43±0.03[a] | 2.10±0.05 | 0.40±0.01[ab] | 0.37±0.01[b] | 0.40±0.02[ab] |
| 2 | 937 | α-Pinene | 2.84±0.24[a] | 0.68±0.01 | 1.74±0.14[b] | 1.60±0.03[b] | 1.68±0.04[b] |
| 3 | 946 | Camphene | 1.55±0.11[a] | ----- | 1.55±0.12[a] | 1.42±0.05[a] | 1.50±0.06[a] |
| 4 | 977 | Sabinene | 8.31±0.30[a] | 1.50±0.01 | 8.36±0.25[a] | 7.72±0.12[b] | 7.95±0.15[ab] |
| 5 | 982 | β-Pinene | 3.02±0.15[a] | 0.32±0.00 | 1.99±0.09[b] | 1.84±0.09[b] | 1.93±0.10[b] |
| 6 | 991 | Myrcene | ----- | 1.97±0.10 | ----- | ----- | ----- |
| 7 | 1009 | α-Phellendrene | ----- | 0.34±0.01 | 0.05±0.00[a] | ----- | ----- |
| 8 | 1021 | α-Terpinene | 0.19±0.01[a] | 5.60±0.15 | 0.23±0.01[bc] | 0.22±0.00[b] | 0.25±0.01[c] |
| 9 | 1024 | ρ-Cymene | ----- | 4.74±0.04 | ----- | ----- | ----- |
| 10 | 1034 | D-Limonene | 71.50±0.50[a] | 1.82±0.05 | 66.60±1.20[b] | 69.98±0.90[a] | 69.86±0.60[a] |
| 11 | 1064 | γ-Terpinene | 5.58±0.02[a] | 18.15±0.10 | 9.89±0.50[b] | 9.32±0.20[b] | 9.33±0.25[b] |
| 12 | 1074 | Z-Sabinene hydrate | ----- | 1.65±0.02 | ----- | 0.06±0.00[a] | 0.06±0.00[a] |
| 13 | 1095 | E-Sabinene hydrate | ----- | 1.35±0.01 | 0.47±0.03[a] | 0.44±0.01[a] | 0.45±0.02[a] |
| 14 | 1103 | Terpinolene | 0.42±0.01[a] | 0.36±0.01 | ----- | ----- | 0.15±0.01[b] |
| 15 | 1107 | Linalool | ----- | 7.32±0.05 | 0.09±0.00[a] | 0.10±0.01[b] | 0.17±0.01[c] |
| 16 | 1110 | β-thujone | ----- | 0.27±0.01 | ----- | ----- | ----- |
| 17 | 1129 | E-ρ-2-Menthen-1-ol | ----- | 0.74±0.03 | ----- | ----- | ----- |
| 18 | 1147 | Camphor | ----- | 0.41±0.01 | 0.09±0.00[a] | 0.08±0.00[a] | 0.09±0.00[a] |
| 19 | 1188 | α-Terpineol | 0.18±0.02[a] | 11.00±0.20 | 0.09±0.00[b] | 0.12±0.00[c] | 0.15±0.00[a] |
| 20 | 1200 | E-Piperitol | ----- | 5.53±0.03 | 0.24±0.01[a] | 0.24±0.01[a] | 0.26±0.01[a] |
| 21 | 1245 | Neral | 0.69±0.05[a] | ----- | 0.87±0.02[b] | 0.79±0.04[b] | 0.70±0.05[a] |
| 22 | 1274 | Geranial | 1.15±0.13[a] | ----- | 1.38±0.06[b] | 1.26±0.04[ab] | 1.18±0.02[a] |
| 23 | 1305 | Thymol | ----- | 30.53±0.10 | 0.10±0.00[a] | 0.16±0.00[b] | 0.21±0.00[c] |
| 24 | 1313 | Carvacrol | ----- | 1.59±0.01 | 0.30±0.02[a] | 0.46±0.02[b] | 0.65±0.04[c] |
| 25 | 1363 | Neryl acetate | 0.36±0.05[a] | ----- | 0.51±0.02[b] | 0.50±0.05[b] | 0.44±0.01[ab] |
| 26 | 1382 | α-Copaene | 0.23±0.01[a] | ----- | 0.22±0.01[ab] | 0.20±0.00[bc] | 0.19±0.01[c] |
| 27 | 1434 | Germacrene D | 0.28±0.03[a] | 0.77±0.01 | 0.34±0.01[b] | 0.30±0.01[ab] | 0.28±0.01[a] |
| 28 | 1510 | δ-Cadinene | ----- | 0.25±0.00 | 0.08±0.00[a] | 0.06±0.00[a] | 0.66±0.03[b] |
| 29 | 1514 | β-Bisabolene | 0.76±0.06[a] | ----- | 0.82±0.04[a] | 0.75±0.02[a] | ----- |

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| | | | | | |
|---|---|---|---|---|---|
| Total | 97.49±1.72[a] | 98.99±1.01 | 96.41±2.54[b] | 97.99±1.61[c] | 98.54±1.44[d] |

[a] Retention indices of volatile compounds on DB-5 columns. [b] Compounds listed according to their elution on DB-5 column. [c] Value (average of triplicate determinations) expressed as relative area percentages to total identified compounds ± Standard deviation. Mean values in the same row for lemon EO and its blends with oregano EO followed by different superscript lower case letters are significantly different at $P < 0.05$.

### 3.2 Radical scavenging activity

The DPPH and ABTS radical assay were used for the evaluation of the radical scavenging activity of lemon oil, oregano EO and their blends (Fig.1 a, b). The moderate scavenging ability of lemon oil may be correlated to the presence some antioxidant compounds such as γ-terpinene and terpinolene [28], alcohol terpines [29] and citral isomers (neral and geranial) [8]. The high inhibition of the free radical DPPH by oregano EO is mainly attributed to the presence of the phenolic compound thymol, major identified compound, and its isomer carvacrol [30] in addition to the synergistic interaction among other antioxidant compounds such as ρ-cymene, γ-terpinene, terpinolene and α-terpineol [19].

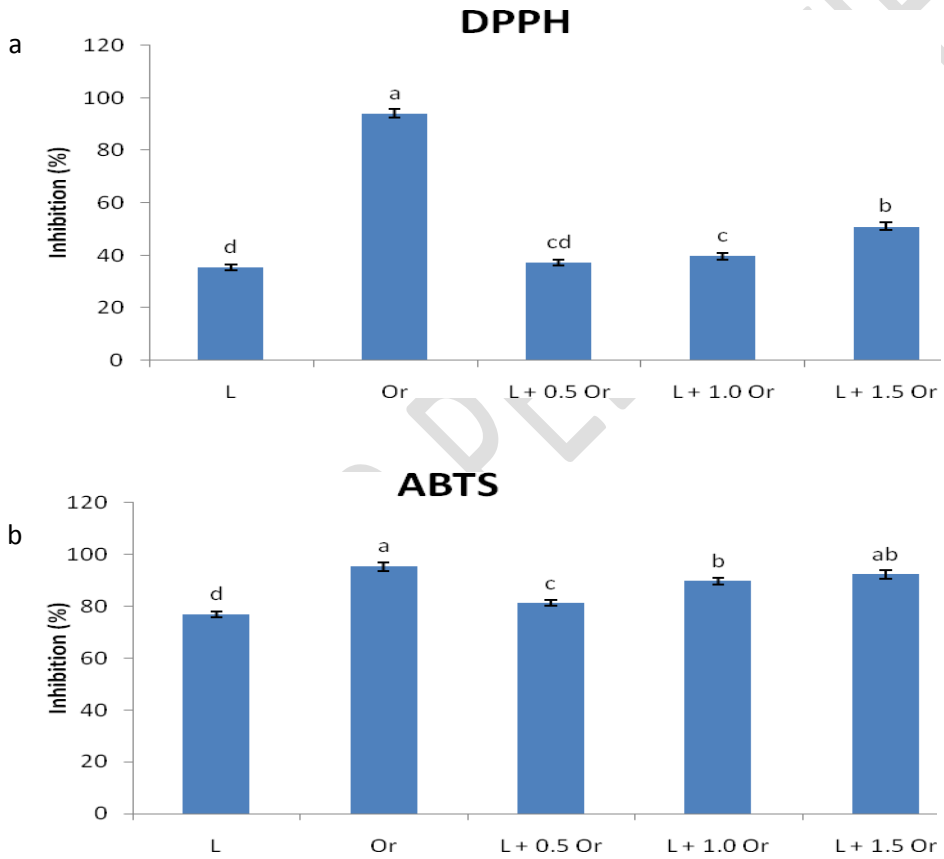


**Fig. 1. Radical scavenging activity of lemon (L) oil and its blends with oregano essential oil (Or).**

Values followed by different superscript letters are significantly different at $P < 0.05$

### 3.3 Odour sensory evaluation and radical scavenging activity of lemon-oregano blends

Blend of lemon oil with oregano EO (0.5, 1.0, 1.5 and 2 µl oregano EO / 100 µl lemon oil) were prepared and subjected to odour sensory evaluation (Table 2). It is obvious that increasing the added amount of oregano EO to lemon oil up to 1.5 µL /

100 µL resulted in a gradual improvement in odour acceptability followed by a significant decrease by addition of 2 µL oregano EO. The improvement of the odour quality may be correlated to the increase in linalool content which possesses floral, rosy, fruity, citrus aroma [31].

As shown in Fig. 1 a and b, the addition of 0.5, 1.0 and 1.5 µl oregano EO/ 100 µl lemon oil improved the radical scavenging activity of lemon oil by 5.5, 12.00 and 44.30%, respectively. This finding may be correlated to the increase in the antioxidant compounds such as α-terpinene, γ-terpinene, linalool, camphor, neral, geranial, thymol and carvacrol (Table 1). As shown in Table 2, the blend containing 2 µL oregano EO showed the least odour score. Therefore, this sample was excluded and not subjected to AOA and GC-MS analysis.

**Table 2. Odour acceptability of lemon oil blended with oregano essential oil at different concentration**

| Blends | Constituents | Odour acceptability |
|---|---|---|
| Control | Lemon oil+ Oregano oil 100 µL + 0.00 µL | $8.0 \pm 0.40^{bc}$ |
| Blend 1 | Lemon oil + Oregano oil 100 µL + 0.50 µL | $8.2 \pm 0.41^{ab}$ |
| Blend 2 | Lemon oil + Oregano oil 100 µL + 1.00 µL | $8.5 \pm 0.42^{ab}$ |
| Blend 3 | Lemon oil + Oregano oil 100 µL +1.50 µL | $9.3 \pm 0.47^{a}$ |
| Blend 4 | Lemon oil + Oregano oil 100 µL +2.00 µL | $7.00 \pm 0.50^{c}$ |

Mean values followed by different superscript lower case letters are significantly different oils $P < 0.05$.

### 3.4 Release of volatile compounds from thickened model system solutions

To illustrate effect of the thickeners (xanthan, Arabic gum and pectin) on release of the volatile compounds from the thickened viscous model systems, it was important first to detect their release from an aqueous solution. The absence of the two oxygenated terpenes linalool and α-terpineol in the volatiles of the aqueous solution (Table 1) may be due to their low contents in the selected L-Or blend or to their retention in water because of their hydrophilic properties [11]. Release of limonene from water comprised 71% of the total volatiles release. This may be correlated to its hydrophobic property that causes its hard retention in water [11]. In general, the release of the total volatile compounds from the thickened model solutions showed a significant decrease ($P < 0.05$) compared to their release from the unthickened model system (Table 3). Fig. 2 revealed that increasing the concentration of each thickener led to a gradual increase in the viscosity of the model solutions. As shown in Fig. 2, xanthan showed much more pronounced thickening properties. The viscosity of the model solution thickened with the lowest concentration of xanthan (0.05 g/ 100 mL water) was higher than that of the model solutions thickened with the highest concentration of Arabic gum (10 g/ 100 mL water) and pectin (0.6 g/ 100 mL water). Mass transfer theory [32] stated that increasing the matrix viscosity would decrease the release of the volatile compounds. In the present study, no linear correlation was found between the release of the total volatiles from each thickened solution, relative to their release from unthickened solution, and increasing its viscosity (Fig. 2).

This result implies that the release of the volatiles from a thickened solution was affected by other factors rather than the viscosity such as compound-compound interaction and compound-thickeners interaction [14,33]. In present study, the content of the target volatile compounds (limonene, neral and geranial) and total content of

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the volatile compounds were considered as response variables in the optimization study.

The influence of xanthane on release of the volatile compounds from thickened solutions containing 0.05, 0.1, 0.2, 0.4 and 0.6 g xanthan / 100 mL water, is shown in Table 3. The target compounds (limonene and citral) showed the highest release in sample thickened with 0.1 g xanthan /100 mL water. The significant ($p < 0.05$) decrease in release of limonene from the thickened solutions compared to that from the unthickened solution is correlated to the existence of certain xanthan- flavour compounds interactions and compounds- compounds interactions [34]. The presence of the hydrophobic compounds such as oxygenated terpines could enhance the aqueous stability of limonene [11]. Previous study indicated that xanthan has a more distinctive hydrophobic character compared to the other hydrocolloids [35] and thus it has the ability to form an internal hydrophobic region and thus prevents the release of hydrophobic volatile compounds [36]. As shown in Table 3, xanthan gum positively affected the release of citral (neral and gerneal). Citral showed higher release (0.25-0.28x$10^6$) from solutions thickened with 0.05-0.20 g xanthan / 100 mL water than its release from unthickened solution (0.22x$10^6$). This result is in agreement with Mirhosseini et al. [14] who reported that the release content of citral from xanthan solution was positively affected by the content of xanthan. Nevertheless, the release of the target compounds was significantly decreased by increasing the xanthan content (0.6 g / 100 mL water). As shown in Table 3, the release of the volatile compounds from solutions thickened with Arabic gum showed the same pattern as xanthan. In a previous study [14], the release of the volatile compounds of orange oil from orange beverages thickened with xanthan and Arabic gum, separately, showed almost the same trend. The authors concluded that the release pattern of the target volatile compounds was significantly governed by the concentration levels of the hydrocolloid rather than their type. However, there were noticeable variations in release content of the volatile compounds between the two thickeners (Table 3). This may be correlated to the physicochemical characteristics of the volatile compounds. The high tendency of Arabic gum to suppress the release of the volatile compounds is correlated to its molecular structure that consisting of Arabinogalactan attached to a polypeptide backbone. The arabinogalactan is able to suppress the release of the hydrophilic compounds whereas, the hydrophobic polypeptide chain has a tendency to bind the hydrophobic compounds [14]. The solution thickened by 0.05 g pectin /100 mL water showed a slight decrease in release of limonene and total volatiles (Table 3) compared with unthickened solution. Whereas, it showed a significant decrease in release of citral (neral and geranial). However, increasing pectin concentration from 0.1 to 0.6 gave rise to a significant decrease in release of limonene and a significant increase in release of citral. Hansson et al. [20] and Boland et al. [33] attributed the great release of the hydrophilic volatile compounds from pectin gels and the opposite behavior of hydrophobic compounds to the combined effect of a number of matrix-volatiles interactions.

From the above mentioned results, it is obvious that the simple model solutions thickened separately, with 0.1, 6.0 and 0.05 g / mL xanthan, Arabic gum and pectin, respectively, showed the highest release of the target volatile compounds and total volatile compounds (Table 3). To confirm these findings, principle component analysis PCA was performed based on the released volatiles in the headspace of the simple model solutions thickened with xanthan, Arabic gum and pectin at variable concentrations. The score plot is shown in Fig. 3. The two first principle components

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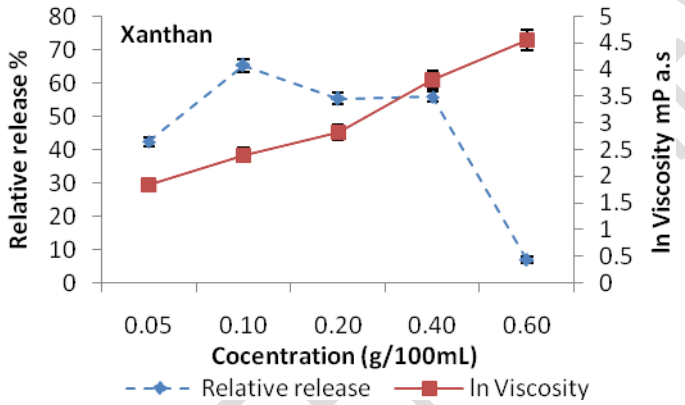
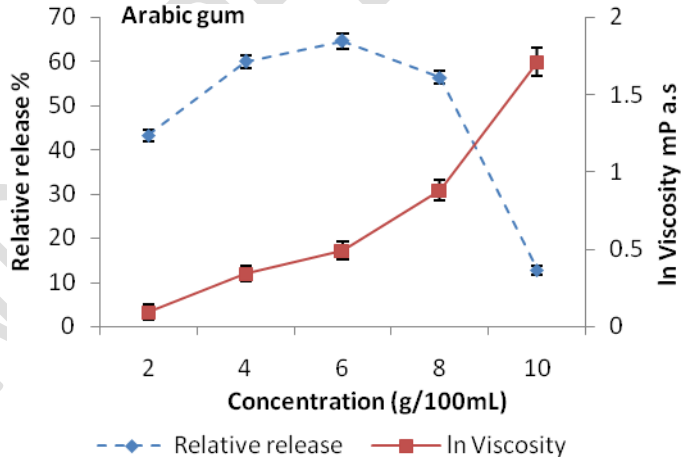
PC1 and PC2 accounted for 86.10% of the total variance of which PC1 explained 51.35% variance and PC2 explained 34.75% variance. As shown in the Fig. 3 the thickened model solutions at different concentrations are clearly differentiated. The simple model solutions thickened with 0.1, 0.2 and 0.4 g xanthan / 100 mL were fallen in the +ve PC1 and PC2. However, the model solutions thickened with 0.1 xanthan showed the nearest distance from control sample (unthickened solution) which was fallen in the same location. As shown in Fig. 3, among the model solutions thickened with Arabic gum, that thickened with 6.0 g/ 100 mL was located in the same location as the control sample (the PC1 and PC2). From Fig. 3, it could be seen that the simple model solution thickened with 0.05 g pectin/ 100 mL was located in the +ve PC1. It showed the nearest distance to control sample compared with the other simple solutions thickened with 0.1, 0.2, 0.4 and 0.6 g pectin/ 100 mL which showed a distinct separation from the control sample.

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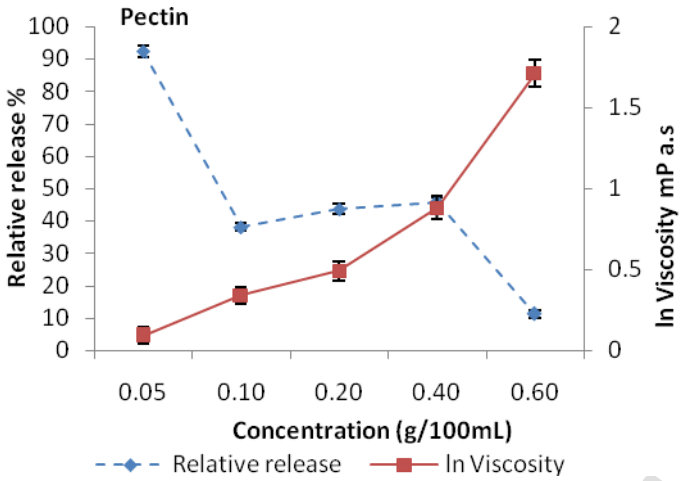


**Fig. 2. Effect of thickeners concentrations on viscosity of the thickened model solutions and release of the volatiles relative to their release from unthickened solution (water)**

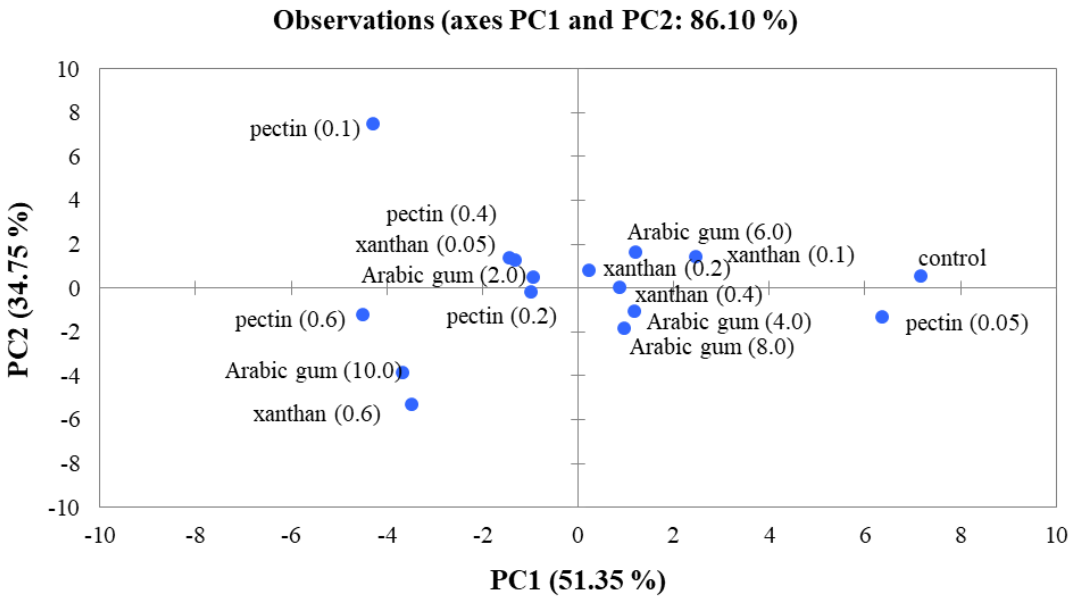


**Fig. 3. Principal Components Analysis (PCA) based on the volatiles released from the thickened simple model systems**

**Table 3. Volatile compounds of the lemon oil-oregano essential oil (L-Or) blend released from simple model solutions thickened with different thickeners**

| Peak No. | Volatile Compounds[a] | Peak area[b] x $10^6$ | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | control | xanthan (g / 100 mL water) | | | | | Arabic gum (g / 100 mL water) | | | | | pectin (g / 100 mL water) | | | | |
| | | | 0.05 | 0.1 | 0.2 | 0.4 | 0.6 | 2 | 4 | 6 | 8 | 10 | 0.05 | 0.1 | 0.2 | 0.4 | 0.6 |
| 1 | α-Thujene | $0.10^{a}$ | $0.02^{b}$ | $0.06^{c}$ | $0.05^{cd}$ | $0.05^{cd}$ | --- | $0.04^{d}$ | $0.05^{c}$ | $0.06^{c}$ | $0.04^{d}$ | $0.01^{b}$ | 0.10a | --- | $0.04^{d}$ | $0.03^{b}$ | $0.01^{b}$ |
| 2 | α-Pinene | $0.42^{a}$ | $0.10^{b}$ | $0.25^{c}$ | $0.22^{cd}$ | $0.22^{cd}$ | $0.04^{e}$ | $0.15^{f}$ | $0.23^{c}$ | $0.23^{c}$ | $0.19^{d}$ | $0.06^{e}$ | $0.40^{a}$ | $0.07^{be}$ | $0.15^{f}$ | $0.12b^{f}$ | $0.03^{e}$ |
| 3 | Camphene | $0.01^{a}$ | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | ---- | 0.01a | ---- | ---- | ---- | ---- |
| 4 | Sabinene | $2.63^{a}$ | $0.63^{b}$ | $1.52^{c}$ | $1.20^{d}$ | $1.21^{d}$ | $0.17^{e}$ | $0.84^{b}$ | $1.32^{cd}$ | $1.42^{cd}$ | $1.16^{d}$ | $0.29^{e}$ | $2.46^{a}$ | $0.50^{b}$ | $0.86^{b}$ | $0.73^{b}$ | $0.20^{e}$ |
| 5 | β-Pinene | $0.81^{a}$ | $0.18^{b}$ | $0.44^{c}$ | $0.33^{cd}$ | $0.34^{ce}$ | $0.04^{f}$ | $0.23^{bde}$ | $0.38^{c}$ | $0.41^{c}$ | $0.33^{ce}$ | $0.06^{bf}$ | $0.76^{a}$ | $0.20^{b}$ | $0.23^{bde}$ | $0.21^{bd}$ | $0.05^{f}$ |
| 6 | α-Phellendrene | $0.02^{a}$ | ---- | $0.01^{b}$ | $0.01^{b}$ | $0.02^{a}$ | ---- | ---- | $0.01^{b}$ | $0.01^{b}$ | $0.01^{b}$ | ---- | $0.02^{a}$ | ---- | ---- | ---- | ---- |
| 7 | α-Terpinene | 0.13a | $0.03^{b}$ | $0.08^{c}$ | $0.05^{d}$ | $0.06^{d}$ | ---- | $0.04^{bd}$ | $0.06^{d}$ | $0.07^{c}$ | $0.06^{d}$ | $0.01^{e}$ | $0.12^{a}$ | $0.03^{b}$ | $0.04^{bd}$ | $0.04^{bd}$ | $0.01^{e}$ |
| 8 | D-Limonene | $23.99^{a}$ | $10.41^{b}$ | $15.38^{c}$ | $13.71^{d}$ | $13.94^{d}$ | $1.72^{e}$ | $10.82^{bd}$ | $14.99^{cd}$ | $15.80^{cd}$ | $14.33^{d}$ | $3.05^{e}$ | $22.26^{a}$ | $8.80^{b}$ | $10.90^{bd}$ | $11.26^{bd}$ | $2.44^{e}$ |
| 9 | γ-Terpinene | $4.87^{a}$ | $2.04^{b}$ | $3.51^{c}$ | $2.29^{b}$ | $2.25^{b}$ | $0.20^{d}$ | $1.70^{b}$ | $2.53^{b}$ | $2.87^{bc}$ | $2.43^{b}$ | $0.37^{d}$ | $4.41^{ac}$ | $1.60^{b}$ | $1.80^{b}$ | $2.09^{b}$ | $0.30^{d}$ |
| 10 | E-Sabinene hydrate | $0.23^{a}$ | $0.13^{b}$ | $0.19^{c}$ | $0.12^{be}$ | $0.12^{be}$ | $0.01^{d}$ | $0.09^{e}$ | $0.13^{b}$ | $0.16^{bc}$ | $0.13^{b}$ | $0.02^{d}$ | $0.21^{ac}$ | $0.11^{bf}$ | $0.10^{be}$ | $0.13^{b}$ | $0.02^{d}$ |
| 11 | Terpinolene | $0.05^{a}$ | $0.04^{b}$ | $0.05^{a}$ | $0.03^{c}$ | $0.03^{c}$ | ---- | $0.03^{c}$ | $0.03^{c}$ | $0.04^{b}$ | $0.03^{c}$ | ---- | $0.04^{b}$ | $0.04^{b}$ | $0.03^{c}$ | $0.04^{b}$ | $0.02^{d}$ |
| 12 | Camphor | $0.03^{a}$ | $0.03^{a}$ | $0.03^{a}$ | $0.02^{b}$ | $0.02^{b}$ | ---- | $0.02^{b}$ | $0.02^{b}$ | $0.02^{b}$ | $0.02^{b}$ | ---- | $0.02^{b}$ | $0.03^{a}$ | $0.02^{b}$ | $0.02^{b}$ | ---- |
| 13 | E-Piperitol | ---- | ---- | $0.01^{a}$ | $0.01^{a}$ | ---- | ---- | ---- | ---- | ---- | 0.01a | ---- | ---- | $0.02^{b}$ | $0.01^{a}$ | $0.01^{a}$ | $0.01^{a}$ |
| 14 | Neral | $0.10^{ac}$ | $0.11^{ab}$ | $0.13^{b}$ | $0.11^{ab}$ | $0.08^{ac}$ | $0.04^{d}$ | $0.12^{ab}$ | $0.09^{ac}$ | $0.11^{ab}$ | $0.06^{c}$ | $0.06^{c}$ | $0.07^{c}$ | $0.17^{e}$ | $0.09^{ac}$ | $0.10^{ac}$ | $0.08^{ac}$ |
| 15 | Geranial | $0.12^{a}$ | $0.14^{a}$ | $0.15^{a}$ | $0.14^{a}$ | $0.11^{a}$ | $0.05^{a}$ | $0.15^{a}$ | $0.10^{a}$ | $0.14^{a}$ | $0.07^{a}$ | $0.07^{a}$ | $0.08^{a}$ | $0.23^{b}$ | $0.11^{a}$ | $0.13^{a}$ | $0.10^{a}$ |
| 16 | Thymol | $0.02^{a}$ | $0.02^{a}$ | $0.02^{a}$ | $0.02^{a}$ | $0.02^{a}$ | ---- | $0.03^{b}$ | ---- | $0.03^{b}$ | $0.01^{a}$ | ---- | ---- | $0.04^{b}$ | $0.02^{a}$ | $0.03^{b}$ | $0.02^{a}$ |
| 17 | Neryl acetate | $0.05^{a}$ | $0.09^{b}$ | $0.05^{a}$ | $0.07^{c}$ | $0.08^{bc}$ | $0.02^{d}$ | $0.07^{c}$ | 0.06ac | $0.09^{b}$ | $0.03^{d}$ | $0.03^{d}$ | $0.03^{d}$ | $0.19^{e}$ | $0.05^{a}$ | $0.06a^{c}$ | $0.04^{a}$ |
| 18 | α-Copaene | ---- | $0.03^{a}$ | $0.02^{b}$ | $0.03^{a}$ | $0.03^{a}$ | $0.01^{c}$ | $0.03^{a}$ | $0.02^{b}$ | $0.03^{a}$ | $0.01^{c}$ | $0.01^{c}$ | $0.01^{c}$ | $0.07^{d}$ | $0.02^{b}$ | $0.02^{b}$ | $0.02^{b}$ |
| 19 | Germacrene D | $0.05^{a}$ | $0.12^{ac}$ | $0.07^{a}$ | $0.08^{a}$ | $0.10^{a}$ | $0.01^{b}$ | $0.07^{a}$ | $0.06^{a}$ | $0.11^{a}$ | $0.05^{a}$ | $0.05^{a}$ | $0.03^{b}$ | $0.28^{d}$ | $0.09^{a}$ | $0.14^{ac}$ | $0.08^{a}$ |

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| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 20 | δ-Cadinene | ---- | 0.03[a] | ---- | 0.02[b] | 0.02[b] | ---- | 0.01[c] | 0.01[c] | 0.03[a] | ---- | 0.03[a] | 0.01[c] | 0.08[d] | 0.02[b] | 0.04[f] | 0.05[e] |
| 21 | β-Bisabolene | 0.05[a] | 0.14[b] | 0.07[ac] | 0.11[bc] | 0.10[c] | 0.03[a] | 0.11[bc] | 0.11[bc] | 0.12[bc] | 0.05[a] | 0.16[b] | 0.04[a] | 0.39[d] | 0.12[bc] | 0.20[f] | 0.28[e] |
| | Total | 33.68[a] | 14.29[b] | 22.04[c] | 18.62[be] | 18.80[be] | 2.34[d] | 14.55[b] | 20.20[ce] | 21.75[ce] | 19.02[e] | 4.28[d] | 31.08[a] | 12.85[b] | 14.70[be] | 15.40[be] | 3.76[d] |

[a] Compounds listed according to their elution on DB-5 column. [b] Values × $10^6$, expressed as total ion chromatograms(TIC) of GC-MS (n = 3) ± Standard deviation. Values followed by different superscript letters in the same row for each thickener are significantly different at $P < 0.05$.

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### 3.5 Release of volatiles from the lemon soft drink

A mixture (2.05 g) of the thickeners (xanthan, Arabic gum and pectin) at the levels that showed the highest release of target compounds (0.1, 6.0 and 0.05 g/ 100 mL water, respectively) were used in formulation of the lemon soft drink. Sucrose, as a sweetener, was used at the level that showed the highest acceptability by panelists in a preliminary study. The viscosity of the formulated soft drink was 3.28 ± 0.16 mPa.s. The headspace volatiles of the formulated soft drink were subjected to SPME and GC-MS analysis. The percentages of the target compounds (limonene and citral) in total identified compounds were calculated and compared, separately, with those of the simple model systems solutions thickened with xanthan, Arabic gum and pectin. As shown in Fig. 4, the percentage of limonene in the headspace volatiles of the three model systems was approximately similar (72.1, 72.6 and 71.2%, respectively). The increase in limonene percentage (75.4%) in the headspace volatiles of the lemon soft drink, compared to the model systems, may be correlated to the interaction effect between the three thickeners. These results are in agreement with those of Mirhosseini et al. [14] who correlated the increase in the release of monoterpene hydrocarbons from orange beverage to the interaction between xanthan and Arabic gum. Increase the release of citral (Fig. 4) from the lemon soft drink may be due to the salting out effect of sucrose [20]. The competition between sucrose and volatile compounds in water hydration may give rise to the increase in release of some oxygenated compounds [37]. Furthermore, sucrose/sucrose interactions *via* hydrogen bonds [38] were shown to influence molecular mobility.

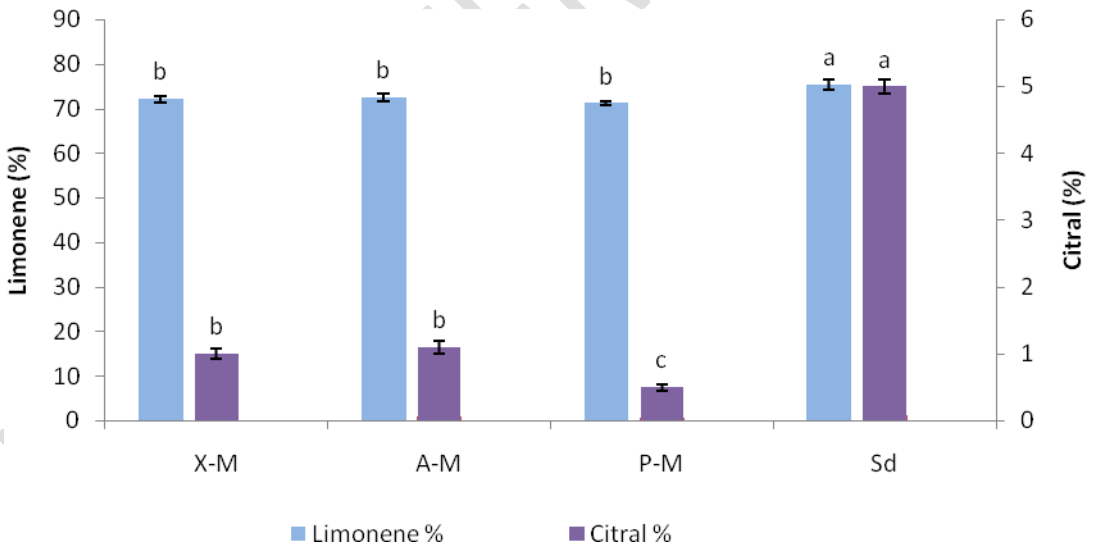


**Fig. 4. The percentages of limonene and citral in the headspace volatiles of soft drink (Sd) and model solutions thickened with xanthan (X-M), Arabic gum (A-M) and pectin (P-M).** Values followed by different superscript letters for limonene % or citral % are significantly different at $P < 0.05$

### 3.6 The physicochemical and sensory characteristics of the carbonated and non carbonated soft drink

The concentration of $CO_2$ dissolved in the soft drink was 1.92 g / L. The effect of carbonation on the values of several chemical parameters of the lemon soft drink is shown in Table 4. As expected the acidity showed a significant ($p < 0.05$) increase while the pH value showed an opposite trend. This result may be ascribed to the dissociation of carbonic acid during titration [39,40]. The total soluble solids (TSS) and total sugar showed insignificant ($P > 0.05$) changes. This result is in agreement with previous studies that showed a slight difference in TSS between the carbonated and noncarbonated juices [39]. Regarding the sensory characteristics, except for colour, the carbonation process improved all the investigated sensory attributes (Fig. 5). It well documented that $CO_2$ gas imparts a distinctive taste, zest and sparkle to the beverage [41]. The results obtained in present study are in agreement with those of previous studies [39,42,43].

**Table 4. Physicochemical characteristics of the carbonated and uncarbonated lemon soft drink**

| Parameters | Soft drink | |
|---|---|---|
| | uncarbonated | carbonated |
| pH | 5.00±0.02[a] | 4.15±0.02[b] |
| Acidity (%) | 0.32±0.01[a] | 0.55±0.02[b] |
| Brix (%) | 14.7±0.10[a] | 14.6±0.10[a] |
| Total sugar (mg / L) | 8.25± 0.02[a] | 8.20±0.03[a] |

Values followed by different superscript letters in the same row are significantly different at $P < 0.05$.

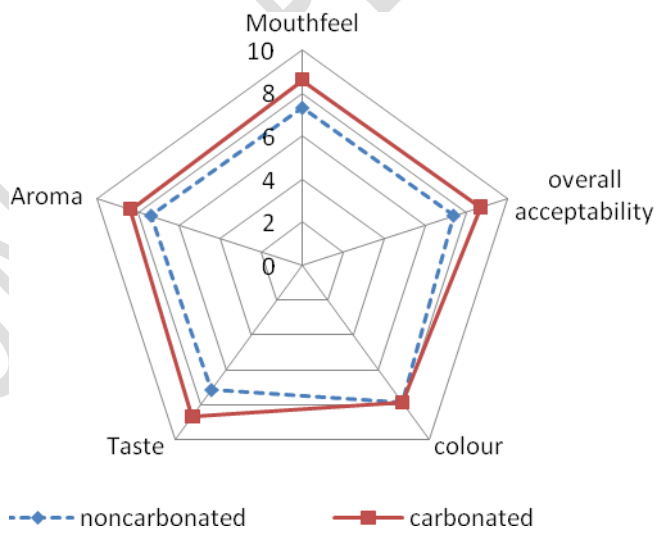


**Fig. 5. The sensory characteristics of the carbonated and uncarbonated lemon soft drink**

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## 4. CONCLUSION

Carbonated lemon soft drink is one of the most popular beverages. Therefore, it was interesting to improve its functional properties such as antioxidant. Blending lemon essential oil (L) with oregano (Or) essential oil (1.5µL Or /100 µL Lemon) improved the odour acceptability and antioxidant activity of lemon essential oil. The selected blend (L-Or) was used in preparation of simple model systems thickened with different thickeners. The model systems thickened with xanthan, Arabic gum and pectin (0.1 g, 6.0 g, 0.05 g/ 100 mL water, respectively) showed the highest release of the target compounds (limonene and citral) and total volatiles. Lemon soft drink was formulated with thickeners at the levels that showed the highest release of the target compounds. The significant increase in percentage of the target compounds in total headspace volatiles of the soft drink compared to that in each model system was correlated to the thickener-thickener interaction and compound-thickener interaction. Carbonation of the soft drink resulted in a significant increase in the overall acceptability and acidity and a decrease in pH value. The prepared carbonated lemon soft drink is expected to be beneficial for health conscious population.

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