GC-MS-Based Prediction of Flavor Compounds in Some Non-Alcoholic Beverages Soft Drink in the Egyptian Market

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Abstract: Recently, some non-alcoholic beverages such as Fanta, Miranda and Schweppes pomegranate have been especially successful in the marketplace as soft drinks and cocktails. Such beverages have been developed to have a more premium taste, with stronger fruit or other base flavors. Hence, to develop such products in the food and beverage market, it is of importance to clearly differentiate them from competing products and identification the aroma compounds existing. In generally, the evaluation of volatiles compounds in food is an important aspect of food production processes. It gives some knowledge about the quality of foods and their relationship to consumers’ choices. Unraveling variation on the profile aroma compounds of non-alcoholic soft drinks for different trademark by GC-MC head space analysis. The objective of the current study is to investigate the aroma profile and major flavor compounds in some non-alcohols soft drinks that have a unique flavor and relative concentrations in these beverages to characterize the odor-active compounds and distinguished their trademarks by GC-MS analysis. Volatile components extract of famously three trademarks from non-alcoholic soft drinks (FT, MD and SP) were quantitatively and qualitative analyzed using gas chromatographic (GC) techniques. 11 compounds were identified and qualitative with using GC-MS. Analytical experiments showed that the critical compounds that contribute to the characteristic flavor of the studied non-alcoholic soft drinks were as the following : (1) D-Limonene, (2) alpha-Terpineol, (3) beta-Myrcene, (4) Cyclohexene, 1-methyl-4-(1-methylethylidene)-(5)2-Carene,(6) Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1,alpha.,2.beta.,5.alpha)-,(7) gamma.-Terpinene,(8) (1R,2S,4R)-2,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol,(9) 3-Cyclohexen-1-ol, 5-methylene-6-(1-methylethenyl)- acetate,(10) 2-Pentadecyn-1-ol, and (11)2-Decyn-1-ol. Major volatile compound of the commercial non-alcoholic soft drinks (FT, MD and SP) was detected as limonene (80.54, 84.54 and 95.65, respectively). All the 11 compounds identified are the primary constituents of the flavor components in the tested three trademarks non-alcoholic soft drinks in Egyptian market.

Key words: Aroma compounds • Non-alcoholic soft drinks • GC/MS • Validation method • beta.-Myrcene • D-Limonene • Cyclohexene • Egyptian standard specifications

INTRODUCTION

In recent years, gas chromatography olfactometry (GC-O), solid-phase micro extraction (SPME), and gas chromatography/mass spectrometry (GC/MS) have been widely applied to the research on the aroma compounds in liquor Wang et al. [1] and Chinese researchers have conducted a number of in-depth studies of the strong-favor, Daqu fen-favor, and Maotai-favor liquor.

For example, Wang et al. [1] have investigated the major components in Maotai-favor liquor by liquid-liquid extraction and GC/MS and carried out quantification analysis of 48 aroma compounds and finally, 29 compounds were identified as the skeleton components, and the results were validated by aroma recombination and omission experiments [2].

Fan et al.[3] conducted a quantitative analysis of 66 favor compounds in soft-style liquor with Head Space Solid Phase Micro-extraction (HS SPME) and identified the key favor compounds such as ethyl hexanoate and 2-methyl ethyl butyrate by calculating the odor activity values (OAVs) [4].

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Agreeing with the opinion of Liberto et al. [5] the headspace (HS) is the best technical mode to picture the volatile compounds profile because it is an equilibrium between the vapor phase and food matrix. Therefore, the most widely used in food analysis is the HS-SPME.

At the GC injection port, the analysts are thermally desorbed and then transferred to the chromatographic column using a carrier gas for their separation [6].

The success of the SPME method might be connected with its timesaving feature and possible options for automatization, as well as non-solvents usage [7]. The positive aspect of the SPME usage is that it can cover multiple steps of analysis such as sampling, separation of compounds of interest from the other matrix compounds, transfer of analyses from outside to the laboratory, and transport of analyses to the instrument.

As said by Jelen et al. [8], the main classes of food products that the SPME was implemented for aroma analysis are: beverages, dairy, fruits/vegetables, honey, meat, seafood, and wine [9].

Moreover, the main problems that were solved by using SPME are: studies of volatiles in different varieties of food products, profiling individual molecules reliable for food aroma, categorization of foodstuffs, also proof their authenticity. Furthermore, analysis of particular compounds responsible for food quality, to screen the technological process influences on aroma properties, screening chemical and biochemical processes related to transformations of aroma molecules.

In addition, Starowicz et al. [10] demonstrated that volatile compounds analysis with SPME is a favorable tool to determine also the quality of functional food and designing the product for consumer’s needs. Xu et al. [11] found out SPME as an appropriate method to separate volatile components from the oil matrix and afterward to determine compounds responsible for oil quality during storage. Furthermore, SPME coupled with GC/MS, and with other techniques of analysis, e.g., ATR-FTIR could be a promising possibility to prove the authenticity of food products [12]. Aceña et al. [13] admitted that in comparison to other extraction methods, SPME is useful to determine more aroma-active regions of analyzed products whereas Berrou et al. [14] showed a high, 90% recovery of volatiles and semi-volatile using SPME technique.

Furthermore, to study the active components of beverages aroma and taste by gas chromatography. The volatiles are mostly separated on GC systems with the support of mass spectrometry (MS, MS/MS) for chemical compound identification the promising part could be a study of taste and aroma compounds by using GC/MS to identification and naming volatile compounds as first time. Besides, can be used to remotely detect volatile compounds released by food and may be able to be used to identify spoiling food in large food warehouses and food safety fields.

Knowing the volatile profile of the soft drinks ingredients helps to adjust and optimize conditions during soft drinks processing and more understand of interference with nutritional elements or drugs in the digestion system of human.

Hence, these studies have greatly expanded to understanding of the chemical composition of flavor component in three trademarks famously. A current study aims to identify the volatile compounds by GC-MS in trademark studied.

The main objective of this work is the extraction of aroma/taste compounds from different samples (soft drinks taken from local markets) by the GC-MS headspace technique.

The study is primarily focused on the following four objectives:

1) To establish a rapid and accurate method for identifying volatile components of non-alcoholic soft drinks;
2) To provide a novel methodology to investigate flavor compounds of various soft drinks;
3) To establish a visualized method for identifying volatile compounds in commercial soft drinks which produced in local market;
4) In order to include and write down the volatile compounds content of soft drinks and set the exact requirements in the Egyptian food standards.

**MATERIALS AND METHODS**

In this study, a three trademark products produced by 3 different manufacturers (FT= Fanta; MD = Miranda and SP= Schweppes pomegranate.) were used to determine the flavor profiles and characteristics of the samples by GC-MS. The studied samples (Cans) were produced in the period from January 2022 and were obtained from the local market. Ultra-pure hydrogen was used as a carrier gas.

**Gas Chromatography-Mass Spectrometry Analysis (HS-GC/MS):** The GC-MS system (Agilent Technologies) was equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories
Network, National Research Centre, Cairo, Egypt. Headspace temperature program: oven temperature 80°C, needle temperature 90°C, transfer line temperature 105°C and incubation time 15 min. The GC was equipped with DB-624 column (30 m x 320 µm internal diameter and 1.80 µm film thickness). Analyses were carried out using hydrogen as the carrier gas at a flow rate of 2ml/min at a split 1:5, sample volume in HS vial 5ml, injection volume of 1 µl and the following temperature program: 35°C for 1 min; rising at 10°C /min to 100°C and held for 0 min.; rising at 35°C /min to 250°C and held for 5 min. The injector and detector were held at 230°C. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 20-220. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

RESULTS

The chromatograms of the extracted volatiles from the non-alcoholic soft drinks by using static HS technique are shown on Figure (1-a:k); in addition, list of the volatile compounds are given in (Table 1).

Jeleñ [15] mentioned that GC-MS provides high sensitivity and is routinely used in the target analysis of

(a) FT soft drink

(b) MD soft drink

(c) SP soft drink

Fig. 1a-c: GC/MS chromatographs of the volatiles components of three soft drinks (FT, MD and SP) by using GC-MS/HS technique according to retention time and relative abundance (relative intensity).
Fig. 2. a-i: GC/MS chromatographs of the volatiles components and derivatives by using GC-MS/HS technique according to relative abundance (relative intensity) and m/z: (a): beta.-Myrcene; (b): 2-Carene, (c): D-Limonene; (d): gamma.-Terpinene; (e): Cyclohexene, 1-methyl-4-(1-methylethylidene); (f): Cyclohexanol, 2-methyl-5-(1-methylethenyl)-(1.alpha.,2.beta.,5.alpha.); (g): (1R,2S,4R)-2,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol; (h): alpha.-Terpineol (Miranda drink); and (i): 2-Decyn-1-ol. (Pomegranate Schweppes drink).

Table 1: GC-MS quantitative analysis of three carbonated soft drinks trademarks produced in the Egyptian market.

<table>
<thead>
<tr>
<th>Peak</th>
<th>FT</th>
<th>MD</th>
<th>SP</th>
<th>FT</th>
<th>MD</th>
<th>SP</th>
<th>Flavor compounds</th>
<th>Area %</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.267</td>
<td>8.265</td>
<td>8.267</td>
<td>4.82</td>
<td>4.35</td>
<td>1.94</td>
<td>Beta-Myrcene</td>
<td>100</td>
<td>C_{10}H_{16}</td>
</tr>
<tr>
<td>2</td>
<td>8.57</td>
<td>8.567</td>
<td>ND</td>
<td>1.3</td>
<td>1.01</td>
<td>ND</td>
<td>2-Carene</td>
<td>67</td>
<td>C_{10}H_{18}</td>
</tr>
<tr>
<td>3</td>
<td>8.678</td>
<td>8.676</td>
<td>8.666</td>
<td>80.54</td>
<td>84.54</td>
<td>95.65</td>
<td>D-Limonene</td>
<td>100</td>
<td>C_{10}H_{16}</td>
</tr>
<tr>
<td>4</td>
<td>8.914</td>
<td>8.914</td>
<td>8.914</td>
<td>0.52</td>
<td>1.89</td>
<td>0.07</td>
<td>Gamma-Terpinene</td>
<td>100</td>
<td>C_{10}H_{16}</td>
</tr>
<tr>
<td>5</td>
<td>9.144</td>
<td>9.142</td>
<td>9.144</td>
<td>2.31</td>
<td>1.75</td>
<td>0.13</td>
<td>Cyclohexene, 1-methyl-4-(1-methylethylidene)-</td>
<td>100</td>
<td>C_{10}H_{16}</td>
</tr>
<tr>
<td>6</td>
<td>9.255</td>
<td>9.253</td>
<td>ND</td>
<td>0.3</td>
<td>0.21</td>
<td>ND</td>
<td>3-Cyclohexen-1-ol, 5-methylene-6-(1-methylethenyl)-, acetate</td>
<td>100</td>
<td>C_{10}H_{18}O</td>
</tr>
<tr>
<td>7</td>
<td>9.417</td>
<td>9.417</td>
<td>9.419</td>
<td>0.53</td>
<td>0.54</td>
<td>0.56</td>
<td>2-Pentadecyn-1-ol</td>
<td>100</td>
<td>C_{16}H_{34}O</td>
</tr>
<tr>
<td>8</td>
<td>9.763</td>
<td>9.763</td>
<td>ND</td>
<td>0.61</td>
<td>0.45</td>
<td>ND</td>
<td>Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1.alpha.,2.beta.,5.alpha.)-</td>
<td>100</td>
<td>C_{10}H_{18}</td>
</tr>
<tr>
<td>9</td>
<td>9.902</td>
<td>9.904</td>
<td>ND</td>
<td>0.31</td>
<td>0.23</td>
<td>ND</td>
<td>(1R,2S,4R)-2,7,7-Trimethylbicyclo[2.2.1]heptan-2-ol</td>
<td>100</td>
<td>C_{16}H_{30}O</td>
</tr>
<tr>
<td>10</td>
<td>10.008</td>
<td>10.008</td>
<td>10.01</td>
<td>8.77</td>
<td>5.03</td>
<td>0.65</td>
<td>Alpha-Terpineol</td>
<td>100</td>
<td>C_{10}H_{18}</td>
</tr>
<tr>
<td>11</td>
<td>ND</td>
<td>ND</td>
<td>8.6</td>
<td>ND</td>
<td>1.2</td>
<td>2-Decyn-1-ol</td>
<td>100</td>
<td>C_{16}H_{34}O</td>
<td></td>
</tr>
</tbody>
</table>

Whereas, FT= Fanta; MD = Miranda and SP= Schweppes pomegranate.

Compounds responsible also for wine flavors and off-flavors, and for profiling volatile compounds in wine [16].

As it can be seen from (Table 1) 11 volatile compounds were extracted from non-alcoholic soft drinks using GC-MS HS technique. The percentage of D-Limonene which is one of the major volatiles of the non-alcoholic soft drinks Kendirci and Altug, [17] was detected to cover only 80.54, 84.54 and 95.65% of the total peak area of FT, MD and SP samples, respectively.

Furthermore, 10 compounds were identified and qualitative with using GC-MS HS of FT and MD soft drinks, except for SP soft drinks sample where 6 compounds identified, beside, 1.2% of 2-Decyn-1-ol. Analytical experiments showed that the critical compounds that contribute to the flavor characteristic of the tested non-alcoholic soft drink (three famously trademarks) according to area % were as the following:

1) FT high area percentage were D-Limonene 80.54, followed by alpha-Terpineol 8.77, beta.-Myrcene 4.82, Cyclohexene, 1-methyl-4-(1-methylethylidene)-2.31 and 2-Carene 1.2 %;
2) while MD sample were D-Limonene 84.54, followed by alpha-Terpineol 5.03, beta.-Myrcene 4.35, gamma -terpinein 1.89, 1-methyl-4-(1-methylethylidene)-1.89 and 2-Carene 1.01%.
3) Additionally, SP sample soft drinks were D-Limonene 95.65, followed by beta-Myrcene 1.94 and 2-Decyn-1-ol 1.2%.
This explains the differences of flavors between samples, and helping the producers to development a new products and variety. Otherwise, revealing qualitative of volatile components in each trademarks for first time.

With taken consider to, the family Group Terpenes such as alcohols, compounds examples, hexanol such as myrcene and limonene.

**DISCUSSION**

**Volatile Compounds:** Since, the observed changes in TSS and acidity levels likely could not exist in themselves; it was further evaluated possible changes in the composition of aroma volatiles of non-alcoholic soft drinks.

Mass-to-charge ratio of the electron can be measured in this apparatus by comparing the radius of the purple circle, the strength of the magnetic field, and the voltage on the electron gun (m/z), because, the mass of the molecular ion is equal to the molecular weight of the compound. Thus, the mass-to-charge ratio of the molecular ion is equal to the molecular weight of the compound. Besides, it was detected in the selected samples (FT, MD and SP) a total of 11 volatile compounds and identified by gas chromatography/mass spectrometry in these three trademarks (Table 1). The identification of these volatiles was verified according to their mass spectra and by comparing their calculated RIs with those of databases.

A complete understanding of flavor requires an investigation of the reactants and dynamics of the flavor reaction [18].

For instance, D-limonene contributes lower to the aroma of soft drinks even though it is the organic volatile compound in highest concentration in all three trademarks. However, the oxygenated terpenes, present in low concentrations, are the main compounds responsible for the aroma.

Attaway and Oberbacher, [19] the contribution of chemical compounds to food flavor is best understood when their perception thresholds are known, hence, the current study targeted this issue.

In the current study, only 11 compounds were identified with their odor threshold, while in study of Plotto et al., [20] for instance, the odor threshold of D-limonene (13 700 µg L⁻¹) was much higher than that of other compounds such as linalool (113 µg L⁻¹), myrcene (773 µg L⁻¹), α-pinene (1650 µg L⁻¹) and γ -terpinene (3260 µg L⁻¹).

The high odor threshold of D-limonene is the main reason for its contribution to the final aroma of non-alcoholic soft drinks. Furthermore, the mandarin, orange and grapefruit it could be consider of a main source of limonene species.

Yajima et al. [21] identified 68 volatile components in satsuma mandarin juice using peeled fruit prior to extraction to minimize peel oil in the juice. The main juice volatiles were 3-methylbutan-1-ol, trans- hex-2-enal and hexanal, in addition, Thymol, α-pinene and γ-terpinene.

In the present study, only α-terpinene and γ-terpinene were identified in (Table 1). These compounds derivative from D-limonene, causing of odor threshold (6 µg L⁻¹) [22, 23].

However, Flath and Juan [24] found traces of this alcohol in prickly pear, this compound is predominant in mandarin juices and could be used as quality control parameters in mandarin juices, since contents of α-terpineol and terpinen-4-ol increased in processed juices and their accumulation was negatively correlated with juice acceptability.

Additionally, Anon, [25] observed that 7.79% was limonene and α-terpinolene which gives citrus character, 5.64% was α-pinene which gives pine character, 1.80% was β-myrcene which gives must, balsamic and spice characters, and 1.34% was 2-decenal and 2-octenal which give green. Besides nut and fatty characters of aldehydes were the main groups of HS technique [17].

Gas chromatograph mass spectrometer (GC/MS) systems are especially qualified for detecting volatile compounds; they can be used to detect the type of aroma components contained in samples and their quantities, the sensitivities of the Mass Spectrometer detector (MS) is in the range of 10⁻⁶ to 10⁻⁸ g of solute per second [26].

In particular, 11 crucial compounds were tentatively defined as main compounds due to their high aroma expression intensity and remarkable contribution to the flavor characteristics of three trademarks studied.

However, since non-alcoholic soft drinks are often cold consumed, the flavor when samples are heated for headspace analysis might not be the same as the fragrances experienced at the instant they are actually consumed.

**Evaluation a Potential Risks of Human Health According to Literature Studies:** We will be enough to point out the potential risks of only two compounds, limonene and cyclohexane, as follows:

**Acute Exposure Limit of d-limonene:** The current results of D-Limonene ratio are more than 80 % of total area of each carbonated soft drinks. According to previous literature research, R-(+)-limonene (d-limonene) is a
commonly used flavor additive in food, beverages and fragrances for its pleasant lemon-like odor. Considering its increasing applications, it's necessary to understand toxicological effects and risk associated with its use. R-(+)-limonene is rapidly absorbed in experimental animals and human beings following oral administration. In humans, it gets distributed to liver, kidney, and blood resulting in the formation of metabolites like perillic acid, dihydroperillic acid, limonene-1,8-diol and limonene-1,2 diol. Important toxic effects primarily reported in rodents are severe hyaline droplet nephrotoxicity (only in male rats due to specific protein α2u-globulin; however, this effect isn't valid for humans), hepatotoxicity and neurotoxicity. R-(+)-limonene does not show genotoxic, immunotoxic and carcinogenic effects. Substantial data is available about limonene's stability after treatment with thermal and non-thermal food processing techniques [27].

In a study, Marescotti et al. [28] reported that LC50 values showed that the terpene (i.e., flavoring agent which contained 17% v/v more limonene) increased cytotoxicity in differ lung cell types. However, information about toxicity of metabolites formed and their safe scientific limits is not available. In addition, toxicity of limonene degradation products formed during storage of citrus juices isn't known. Based on all available toxicological considerations, R-(+)-limonene can be categorized as a low toxic additive. More detailed studies are required to better understand interaction of limonene with modern food processing techniques as well as degradation products generated and toxicity arising from such products, for this cause must to obligate to write ratio on product label, notably.

Over and above, the International Agency for Research on Cancer (IARC) [29] has classified d-limonene in Group 3 (not classifiable as to its carcinogenicity to humans) based on a lack of available data on carcinogenicity to humans and limited evidence for carcinogenicity in experimental animals.
Covalently-Bonded Unit Count property of Cyclohexane is 1, besides; canonicalization property is chance to react or chelating with food ingredient or interaction with drug and glowing positives effect.

In addition, The International Agency for Research on Cancer (IARC), [35] reported some chemicals that cause tumours of the urinary tract in rodents such as 1-tert-butoxypropan-2-ol, β- myrcene, furfuryl alcohol, melamine, pyridine, tetrahydrofuran, and vinylidene chloride.

Therefore, it appears to be of great importance for continue to support basic research in the field, besides stimulate trials for those flavor compounds and saving reasonable wealth of data is available to limit negative effects. The 2d structure (Fig. 4) and molecular weight 136.2340 (National Institute of Standards and Technology (NIST), [36]).

Conclusion, the current study provides a theoretical basis for unrevealing the flavor standard of soft drinks and realizing the modernization of soft drinks production. Moreover, research on soft drinks might adhere to the dual guidance about “flavor” and “health” in the future.

The results demonstrate that carbonated beverages share many of the same compounds but the relative abundance of these compounds varies by brand.

For potential risks activities, the future studies must focus on metabolism in different animal models of this volatile components and probability an interference with drugs and food components.

However, more research is needed to investigate other volatile chemical constituents in soft drinks, and to examine the bioavailability of volatile components and unraveling variation on aroma compounds of differenced non-alcoholic soft drinks. In addition, identification and safety studies are needed of these components.

Furthermore, it also enriched our knowledge of non-alcoholic soft drinks about volatile compounds as the first time, that can be used for their profiling and trademarks comparison and provided main cause for the evaluation and regulation of the flavor quality of non-alcoholic soft drinks flavor for assurance of good public health, and obligation to show complete ingredients of content non-alcoholic soft drinks on the label.

Significance Statements: The method can be utilized to analyze aroma compounds in non-alcoholic soft drinks by protocol mentioned in an hours, so that benefits the producers and consumers in the end.

On the basis of the results of this study it may be concluded that to the best of our knowledge the present work is the first trial to identification and quantitative of volatiles compounds in non-alcoholic soft drinks, by GC-MS. Over and above, by calculating the odor activity values (OAVs) of these volatile components, we can in the aroma recombination, and applied it in other beverages.

The results provide some guidance for upgrading the quality control and beverage safety and obligate a company's of describe all contents of product on label on a package as the physical and critical rights to consumers.

Finally, All of these analysis should be taken into consideration to routine verifications of soft drinks quality during production periodically before send it to markets, so that benefits the producers and consumers in the end.

REFERENCES


