Comparative Head Space GC/MS Studies of Different Flavored Moâssel in the Egyptian Market (I)

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ABSTRACT
The current study aimed to compare three different types of Egyptian flavored tobacco (Moâssel) used in Hookah smoking. The samples (Apple, Creamy Strawberry and Mix Grapes) were obtained from Al Dandash company (a famous Tobacco company in Egypt). They were analyzed by Head Space GC/MS. There were great differences among the investigated samples. The identified compounds of the Apple sample showed 34 constituents, which represented (93.13%) of the total compounds. The major one was anethole (30.43%). While, the Creamy Strawberry specimen exhibited 27 recognized compounds, which represented (59.61%) of the sample. The chief constituent was acetic acid (15.83%). Finally, the last sample showed 25 identified constituents, which represented (93.16%) of the total compounds and the main compound was 1,2-propanediol (32.74%) of the constituents.

Keywords: Head Space, GC/MS, Egyptian Flavored Moâssel, Al Dandash Company.

INTRODUCTION
Tobacco smoking is an addictive and lethal habit. Moreover, it harms others through passive inhalation of both adults and children to exhaled and side stream smoke\(^1,2\). While, smoking in pregnancy impairs fetal development and growth, in some cases reach to the point of fetal death\(^3\). Moreover, it causes fires generally reduce economic productivity and social engagement\(^4\). One of tobacco smoking types is Hookah (syn.: water pipe, shisha or bubble bubble), which is an old form of non-cigarette tobacco smoking that has been commonly practiced in the middle Eastern region contains over 4800 different chemicals out of which 69 are carcinogens and several others are tumor promoters\(^5,6,7\). Another study demonstrated that humectants such as glycerol and propylene glycol have added to tobacco products to facilitate processing of the cured tobacco leaf, retain moisture and increase half shelf life\(^8,9\). Furthermore, Cooperation Center for Scientific Research Relative to Tobacco (CORESTA) made experiments focused on the quantitative analysis of these humectants in tobacco and tobacco products\(^10\). Also, CORESTA recommended another method to determine nicotine in tobacco and tobacco products by GC/MS\(^10\). All these data provoked us to make Head space GC/MS analyses on different types of flavored Moâssel used in the Egyptian Hookah.

MATERIALS AND METHODS
Materials
Egyptian flavored Moâssel samples viz., Apple (AFM), Creamy Strawberry (CSFM) and Mix Grapes (MixGFM) were collected from the Egyptian market (June 2016).

These specimens were prepared in Al Dandash Company, Egypt.

Methods
Shimadzu GC/MS with Head Space system provided by FID (Flame Ionization Detector), connected to the Mass Spectrometer Model: QP2010Ultra. Total GLC chromatograms and mass spectra were recorded in the electron impact ionization mode at 70 eV, using ACQ Mode of scan from 35 to 500 m/z in 0.3 s. The used column was 0.25 mm in internal diameter, 30 m length, packed with Rtx-MS and 0.25 μm film thickness. The injected volume was 1.0 μl, using helium as carrier gas at flow rate 40 ml/min. The analyses were carried out at a programmed temperature; the initial temperature was 40 °C (Kept for 2 min), then increased at a rate 30-50 °C to the final temperature 210 °C (kept for 5 min). Injector and detector had the same temperature 230 °C. The total run time was 45 min and split ratio 1:50.

RESULTS
Head Space GC/MS analyses
Identifications of compounds were carried out by direct comparison of retention time and fragmentation patterns with those of reference compounds analyzed under the same conditions\(^11,12\) and quantitation was based on peak area integration.

Apple Flavored Moâssel (AFM)
Head Space GC/MS analysis of AFM showed 49 compounds. The unidentified compounds represented 06.87% (15 compounds) and identified compounds represented 93.13% (34 compounds). The major one was anethole (30.43%).

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Table 1: Identified compounds of AFM from Head Space GC/MS.

| No. | Name                                        | RT<sup>1</sup> | RRT<sup>2</sup> | Base peak  | Relative Area % | M. Weight | M. Formula  |
|-----|---------------------------------------------|----------------|----------------|------------|-----------------|-----------|-------------|
| 1   | Acetone                                     | 0.13           | 0.086          | 43         | 00.27           | 58        | C<sub>3</sub>H<sub>6</sub>O  |
| 2   | 2,3-Butanedione                             | 0.23           | 0.105          | 43         | 00.22           | 86        | C<sub>3</sub>H<sub>8</sub>O  |
| 3   | Acetic acid                                 | 0.17           | 0.107          | 44         | 00.28           | 60        | C<sub>2</sub>H<sub>4</sub>O  |
| 4   | 3-Methyl-1-butanol (Fusel oil)              | 0.31           | 0.188          | 55         | 00.98           | 88        | C<sub>5</sub>H<sub>12</sub>O  |
| 5   | 1,2-Propanediol                             | 0.13           | 0.198          | 45         | 01.37           | 76        | C<sub>4</sub>H<sub>10</sub>O  |
| 6   | Dimethyl acetic acid                        | 0.31           | 0.225          | 43         | 00.78           | 88        | C<sub>5</sub>H<sub>12</sub>O  |
| 7   | Butanoic acid                               | 0.23           | 0.254          | 60         | 00.21           | 88        | C<sub>4</sub>H<sub>10</sub>O  |
| 8   | Furfural                                    | 0.30           | 0.304          | 96         | 00.65           | 96        | C<sub>5</sub>H<sub>10</sub>O  |
| 9   | Ethyl-1-butanol                             | 0.23           | 0.316          | 43         | 00.23           | 102       | C<sub>5</sub>H<sub>12</sub>O  |
| 10  | Ethyl-2-methyl butanoate                    | 0.22           | 0.327          | 57         | 01.64           | 130       | C<sub>5</sub>H<sub>12</sub>O  |
| 11  | E-3-hexen-1-ol                             | 0.25           | 0.331          | 41         | 00.19           | 100       | C<sub>5</sub>H<sub>12</sub>O  |
| 12  | Z-3-hexen-1-ol                             | 0.23           | 0.337          | 41         | 05.19           | 100       | C<sub>5</sub>H<sub>12</sub>O  |
| 13  | n-Hexyl formate                             | 0.22           | 0.358          | 56         | 11.43           | 130       | C<sub>5</sub>H<sub>12</sub>O  |
| 14  | 3-Methylbutyl acetate (syn.: Isoamyl acetate or Isopentyl acetate) | 0.21 | 0.366 | 43 | 00.41 | 130 | C<sub>6</sub>H<sub>12</sub>O  |
| 15  | 2-Methylbutyl acetate                       | 0.21           | 0.370          | 43         | 00.24           | 130       | C<sub>6</sub>H<sub>12</sub>O  |
| 16  | Camphene                                    | 0.40           | 0.475          | 93         | 00.18           | 136       | C<sub>10</sub>H<sub>16</sub>  |
| 17  | Benzaldehyde                                | 0.29           | 0.494          | 77         | 00.53           | 106       | C<sub>6</sub>H<sub>12</sub>O  |
| 18  | Isoamyl propionate                          | 0.25           | 0.509          | 57         | 00.20           | 144       | C<sub>6</sub>H<sub>12</sub>O  |
| 19  | Hexanoic acid                               | 0.30           | 0.533          | 60         | 00.75           | 116       | C<sub>6</sub>H<sub>12</sub>O  |
| 20  | Glycerol                                    | 0.31           | 0.542          | 61         | 00.21           | 92        | C<sub>6</sub>H<sub>12</sub>O  |
| 21  | Z-3-Hexenyl acetate                         | 0.24           | 0.568          | 43         | 01.17           | 142       | C<sub>5</sub>H<sub>12</sub>O  |
| 22  | n-Hexyl acetate                             | 0.20           | 0.578          | 43         | 05.45           | 144       | C<sub>5</sub>H<sub>12</sub>O  |
| 23  | Benzyl alcohol (syn.: Phenylmethanol or Benzenemethanol) | 0.23 | 0.616 | 79 | 09.82 | 108 | C<sub>6</sub>H<sub>12</sub>O  |
| 24  | Benzyl acetate                              | 0.60           | 0.811          | 108        | 00.73           | 150       | C<sub>7</sub>H<sub>14</sub>O  |
| 25  | Menthol                                     | 0.27           | 0.830          | 71         | 04.36           | 156       | C<sub>10</sub>H<sub>20</sub>  |
| 26  | Hexyl butanoate (syn.: Hexyl butyrate)      | 0.27           | 0.854          | 43         | 00.44           | 172       | C<sub>10</sub>H<sub>20</sub>  |
| 27  | Anisaldehyde (syn.: 4-Methoxybenzaldehyde)  | 0.23           | 0.951          | 135        | 00.93           | 136       | C<sub>6</sub>H<sub>12</sub>O  |
| 28  | Anethole                                    | 0.22           | 1.000          | 148        | 30.43           | 148       | C<sub>10</sub>H<sub>18</sub>O |
| 29  | Benzyl butanoate                            | 0.21           | 1.077          | 91         | 00.19           | 178       | C<sub>11</sub>H<sub>20</sub>O |
| 30  | Nicotine                                    | 0.21           | 1.083          | 84         | 00.19           | 162       | C<sub>10</sub>H<sub>18</sub>N<sub>2</sub> |
| 31  | Hexyl hexanoate                             | 0.22           | 1.128          | 43         | 00.22           | 200       | C<sub>12</sub>H<sub>20</sub>O |
| 32  | Vanillin                                    | 0.23           | 1.139          | 151        | 00.99           | 152       | C<sub>7</sub>H<sub>14</sub>O<sub>3</sub> |
| 33  | Ethylvanillin (syn.: Vanilul or 3-Ethoxy-4-hydroxy benzaldehyde) | 0.24 | 1.230 | 137 | 06.75 | 166 | C<sub>16</sub>H<sub>18</sub>O<sub>3</sub> |
| 34  | Dihydro methyl jasmonate                    | 0.29           | 1.466          | 83         | 05.80           | 226       | C<sub>13</sub>H<sub>22</sub>O<sub>3</sub> |

Unidentified compounds 06.87%

Identified compounds 93.13%

| Type of Compound | Percentage |
|------------------|------------|
| Oxygenated       | 92.76%     |
| Nitrogenous      | 00.19%     |
| Hydrocarbons     | 00.18%     |

<sup>1</sup>RT: Retention Time.  <sup>2</sup>RRT: Relative Retention Time.

Creamy Strawberry Flavored Moâssel (CSFM)
The identified compounds are classified into three different classes viz., 92.76% oxygenated, 00.19% nitrogenous and 00.18% hydrocarbons compounds as shown in Figure 1 and enumerated in Table 1. 

Head Space GC/MS analysis of CSFM exhibited 37 compounds. The unidentified compounds represented 40.39% (10 compounds) and identified compounds represented 59.61% (27 compounds). The major one was acetic acid (15.83%). The identified compounds are classified into three diverse classes viz., 57.44% oxygenated, 00.90% nitrogenous and 01.27% hydrocarbons compounds as demonstrated in Figure 2 and listed in Table 2. 

Mix Grapes Flavored Moâssel (MixGFM)
Figure 1: Total GC chromatogram of AFM.

Table 2: Identified compounds of CSFM from Head Space GC/MS.

| No. | Name                                           | RT* | RRT** | Base peak | Relative Area % | M. Weight | M. Formula |
|-----|------------------------------------------------|-----|--------|-----------|-----------------|-----------|------------|
| 1   | Acetic acid                                    | 2.26| 43     | 15.83     |                 |           | D2         |
| 2   | Butanoic acid                                  | 5.10| 60     | 01.49     |                 |           | D2         |
| 3   | Furfural                                       | 6.15| 96     | 00.94     |                 |           | D2         |
| 4   | Ethyl-2-methyl butanoate                       | 6.61| 57     | 01.97     |                 |           | D2         |
| 5   | Ethyl isovalerate                              | 6.71| 88     | 00.46     |                 |           | D2         |
| 6   | Z-3-hexen-1-ol                                | 6.81| 41     | 00.79     |                 |           | O          |
| 7   | Camphene                                       | 9.59| 77     | 00.61     |                 |           | C          |
| 8   | Benzaldehyde                                   | 9.99| 93     | 00.50     |                 |           | O          |
| 9   | Glycerol                                       | 10.48| 43    | 00.94     |                 |           | D2         |
| 10  | Hexanoic acid                                  | 10.71| 60    | 01.85     |                 |           | D2         |
| 11  | Limonene                                       | 12.16| 68    | 00.39     |                 |           | O          |
| 12  | Benzyl alcohol (syn.: Phenylmethanol or        | 12.40| 79    | 03.54     |                 |           | C          |
|     | Benzenemethanol)                               |     |        |           |                 |           |            |
| 13  | Benzyl acetate                                 | 16.40| 108   | 01.58     |                 |           | D2         |
| 14  | Ethyl maltol (syn.: 2-Ethylpyromeconic acid)   | 17.52| 140   | 02.70     |                 |           | O3         |
| 15  | Benzyl butanoate                               | 21.78| 91    | 00.43     |                 |           | D2         |
| 16  | Nicotine                                       | 21.86| 84    | 00.90     |                 |           | D2         |
| 17  | Z-Methylcinamate                               | 22.84| 131   | 00.71     |                 |           | D2         |
| 18  | Vanillin                                       | 23.30| 151   | 00.68     |                 |           | O3         |
| 19  | α-Ionone                                       | 24.00| 121   | 00.96     |                 |           | O          |
| 20  | Ethylvanillin (syn.: Vanilal or 3-Ethoxy-4-hydroxy benzaldehyde) | 24.80| 137   | 04.70     |                 |           | O3         |
| 21  | γ-Decalactone                                  | 25.06| 85    | 02.35     |                 |           | O2         |
| 22  | β-Ionone                                       | 25.56| 177   | 01.22     |                 |           | O2         |
| 23  | δ-Decalactone                                  | 25.80| 99    | 00.89     |                 |           | O2         |
| 24  | γ-Undecalactone                                | 27.70| 85    | 00.92     |                 |           | O2         |
| 25  | α-Amylcninnaldehyde (syn.: Z- Jasminaldehyde)  | 29.50| 129   | 10.21     |                 |           | O          |
| 26  | Dihydro methyl jasmonate                       | 29.61| 83    | 01.67     |                 |           | O3         |
| 27  | Neophytadiene                                  | 33.55| 68    | 00.38     |                 |           | O2         |

|                |                                                |
|----------------|------------------------------------------------|
| Unidentified compounds 40.39% | Oxygenated compounds 57.44% |
| Identified compounds 59.61% | Nitrogenous compounds 00.90% |
|                             | Hydrocarbons compounds 01.27% |

*RT: Retention Time. **RRT: Relative Retention Time.
Head Space GC/MS analysis MixGFM displayed 34 compounds. The unidentified compounds represented 06.84% (9 compounds) and recognized compounds represented 93.16% (25 compounds). The major one was 1,2-propanediol (32.74%). The identified compounds are classified into three various classes viz., 92.51% oxygenated, 00.37% nitrogenous and 00.28% hydrocarbons compounds as shown in Figure 3 and recorded in Table 3.

DISCUSSION

The present study investigated three different Egyptian flavored Moâssels viz., Apple, Creamy Strawberry and Mix Grapes by Head Space GC/MS analyses. The samples showed very high percentage of oxygenated compounds and traces of (nitrogenous & hydrocarbons) constituents. Therefore, they have strong flavored odors. The three samples had six common compounds viz., acetic acid, butanoic acid, furfural, Z-3-hexen-1-ol, benzyl alcohol and nicotine. Furthermore, AFM and CSFM had also ten common compounds viz., ethyl-2-methyl butanoate, camphene, benzaldehyde, hexanoic acid, glycerol, benzyl acetate, benzyl butanoate, vanillin, ethylvanillin and Dihydro methyl jasmonate. But, AFM and MixGFM had another common compound; 1,2-propanediol. Finally, CSFM and MixGFM had also three common
compounds viz., ethyl maltol, α-ionone and neophytadiene. From these data, there are relatively differences between the three studied samples specially between (AFM & MixGFM) and (CSFM & MixGFM). While, AFM and CSFM samples are the most similar. The AFM exhibited that anethole was the main compound (30.43%). It is an organic compound, which was widely used as a flavouring agent, showing a reduction in vitro and in vivo leucocytes migration induced by formyl-methionyl-leucyl-phenylalanne (FMLP), leukotriene B4 (LTB4) and carrageenan11. In addition to, it suppressed cell survival and induced apoptosis in human breast cancer cell independent on estrogen receptor status12. Furthermore, it demonstrated an inhibitory effect in non-immune acute inflammation13. However, it was associated with a slight increase in liver cancer in rats14. Moreover, it was a slightly toxic and irritant substance in large quantities15. While, the CSFM showed that acetic acid (15.83%) was the chief identified constituent. It has many synonyms as ethanoic acid or methane carboxyl acid or ethylic acid or methane carboxylic acid15. It is used in pharmaceutical, plastics and chemical industries. During controlled exposure to vapours of acetic acid, it caused a mild nasal irritation at 10 ppm9. It demonstrated an anticancer activity since the 1800s20. Moreover, it possessed a broad antibacterial spectrum against Streptococi, Staphylococci, Pseudomonas, Enterococci and others21,22. Also, it can treated skin infections caused by Pseudomonas resistant to ideal antibiotics23. Furthermore, it can be also used to treat obesity-linked type 2 diabetic Otsuka Long-Evans Tokushima Fatty rats24. Finally, the third one MixGFM displayed that 1,2-propanediol (32.74%) was the major secondary metabolite. The undiluted 1,2-propanediol was minimally

### Table 3: Identified compounds of MixGFM from Head Space GC/MS.

| No. | Name                                      | RT*  | RRT** | Base peak | Relative Area % | M. Weight | M. Formula |
|-----|-------------------------------------------|------|-------|-----------|-----------------|-----------|------------|
| 1   | 5,6-Epoxy-β-ionone                        | 0.147| 0.359 | 40        | 26.82           | 208       | C13H20O2   |
| 2   | 2-Propanol (syn.: Isopropanol)             | 0.173| 0.423 | 45        | 02.41           | 60        | CH3O      |
| 3   | Acetic acid                                | 0.162| 0.528 | 43        | 01.55           | 60        | C2H4O2    |
| 4   | 1-Hydroxy-2-propanone (syn.: Acetol)       | 0.279| 0.682 | 43        | 00.27           | 74        | C3H6O2    |
| 5   | 2,4-Dimethyl-1,3-dioxolane                 | 0.073| 0.751 | 43        | 00.21           | 102       | C6H10O2   |
| 6   | 1,2-Propanediol                            | 0.094| 1.000 | 45        | 02.41           | 76        | C2H6O2    |
| 7   | Butanoic acid                              | 0.185| 1.267 | 60        | 01.85           | 88        | C4H8O2    |
| 8   | Ethyl butanoate                            | 0.305| 1.296 | 71        | 01.98           | 116       | C5H10O2   |
| 9   | Furfural                                   | 0.146| 1.501 | 96        | 00.77           | 96        | C4H8O2    |
| 10  | Z-3-Hexen-1-ol                             | 0.806| 1.663 | 41        | 01.50           | 100       | C5H10O2   |
| 11  | Propylene glycol 1-acetate (syn.: 1-Acetoxy-2-propanol or 2-Hydroxypropylacetate) | 0.750| 1.834 | 43        | 00.28           | 118       | C10H18O3  |
| 12  | Benzyl alcohol (syn.: Phenyldimethanol or Benzenemethanol) | 12.41 | 3.034 | 79        | 04.33           | 108       | C8H10O    |
| 13  | Butanoic acid anhydride                    | 13.44| 3.286 | 71        | 00.40           | 158       | C8H14O3   |
| 14  | Heptanoic acid                             | 13.78| 3.369 | 60        | 00.33           | 130       | C8H14O3   |
| 15  | Ethyl acetacetate (syn.: Ethyl 3-oxobutanoate) | 14.38 | 3.516 | 43        | 00.23           | 130       | C8H14O3   |
| 16  | 3-Hydroxy-2,3-dihydromaltol (syn.: 2,3-Dihyro-3,5-dihydroxy-6-methyl 4H pyran-4-one) | 15.80 | 3.863 | 43        | 00.32           | 144       | C9H14O4   |
| 17  | Z-3-Hexenyl butyrate                       | 17.10| 4.181 | 67        | 00.63           | 170       | C10H18O2  |
| 18  | Ethyl maltol (syn.: 2-Ethylpyroconeic)     | 17.53| 4.286 | 140       | 00.29           | 140       | C9H18O3   |
| 19  | Phenyl-3-methylbutanoate                   | 21.78| 5.325 | 91        | 00.52           | 178       | C11H16O2  |
| 20  | Nicotine                                   | 21.80| 5.330 | 84        | 00.37           | 162       | C10H14N2  |
| 21  | 3-Allyl-2-methoxyphenol                    | 22.14| 5.413 | 164       | 02.19           | 164       | C11H14O2  |
| 22  | Z-Jasnone                                  | 23.28| 5.692 | 79        | 01.82           | 164       | C11H14O2  |
| 23  | E-β-Damascone                              | 23.70| 5.795 | 177       | 07.46           | 192       | C3H8O2    |
| 24  | α-Ionone                                   | 24.03| 5.875 | 121       | 03.61           | 192       | C10H18O2  |
| 25  | Neophytadiene                              | 33.55| 8.203 | 68        | 00.28           | 278       | C20H34O   |

Unidentified compounds 06.84%
Identified compounds 93.16%

Oxygenated compounds 92.51%
Nitrogenous compounds 00.37%
Hydrocarbons compounds 00.28%

*RT: Retention Time. **RRT: Relative Retention Time
irritating to the eye and producing slight transient conjunctivitis. The eye recovered after the exposure removed\textsuperscript{25}. Its concentration increased the hazard of respiratory and immune ailments in children including asthma, hay fever, eczema and allergies from 50% to 180%\textsuperscript{26,27}.

**CONCLUSION**
By comparing three different samples of Moâssel (Apple, Creamy Strawberry and Mix Grapes) from one of the most popular company in Egypt (Al Dandash Company), showed pronounced difference in the identified constituents. Therefore, it is possible for researchers to predict the physiological effects for these samples.

**CONFLICT OF INTEREST**
We declare that no conflict of interest.

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