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Application of headspace-solid-phase microextraction and HPLC for the analysis of the aroma volatile components of treacle and determination of its content of 5-hydroxymethylfurfural (HMF)

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Abstract

In this investigation, the headspace volatiles of treacle samples from three different producers were subjected to GC-MS and HPLC analysis in order to assess the variation in their aroma volatile composition and their content of 5-hydroxymethylfurfural (HMF). The volatile constituents were rich in aliphatic short chain acids, specially acetic acid, alcohols, aldehydes, ketones, and furan derivatives. The HMF content varied according to the producer from 66 mg/kg to 179 mg/kg. The present investigation is considered to be the first, which determine the volatile aroma profile and HMF content for treacle.

Keywords: Treacle; Black honey volatiles; 5-Hydroxymethylfurfural

1. Introduction

Treacle also known as black honey is prepared by concentrating sugar cane juice obtained from fully matured sugar cane stalks, using heat (EOSQC, 1993). As a result of this treatment, the non-enzymatic heat-induced browning reactions, namely Maillard and caramelisation reactions, take place, leading to the formation of several aroma-active volatile aldehydes, ketones and heterocyclic compounds. These volatiles contribute to the characteristic caramel-like, slightly burnt-sugar aroma and flavour of treacle. For this reason, treacle is used extensively as a natural flavouring agent. It is suited to full-flavoured recipes like puddings, fudges, barbecue sauces, licorice, candy and marinades. Treacle is also prescribed as a rich source of iron (approximately 6%) to those suffering from anaemia (unpublished data).

5-Hydroxymethylfurfural (HMF) is often found as a volatile components of heat-processed food (including treacle). This component has received much attention, due to its suspected health hazards, including cytotoxicity, genotoxicity and mutagenicity (Sommer, Hollnagl, Schneider, & Glatt, 2003; Surh & Tannenbaum, 1994; Ulbricht, Northup, & Thomas, 1984) and many countries have set a maximum limit for HMF content in heated foods. The detection and quantitation of HMF has been reported in different thermally processed foods and beverages (Claeys, Van Loey, & Hendrickx, 2003; Consonni & Gatti, 2004; Driffield et al., 2005; Li & Lu, 2005; Pichler & Murkovic, 2004; Shinoda, Komura, Homma, & Murata, 2005).

Despite the diverse applications of treacle in the food industry, no investigations, so far, have tried to identify the aroma volatile components responsible for its characteristic flavour. At the same time no reports on the presence of HMF in treacle are available.

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2. Materials and methods

2.1. Treacle samples

Three treacle samples from different producers were purchased from the local market in Cairo, Egypt. The samples were within their shelf life period.

2.2. Isolation of the aroma volatiles from treacle using headspace-solid-phase microextraction (HS-SPME)

Two hundred milligram of each treacle sample were put into a headspace vial. The samples were equilibrated for 5 min at 35 °C, while stirring thoroughly using a magnetic stirrer. An automated SPME sampler (Combi PAL; CTC Analytics, Zwingen, Switzerland) with a 2-cm stable-flex fibre assembly (Supelco, Bellefonte, PA) coated with 50/30 μm divinylbenzene/Carboxen™/polydimethylsiloxane (DVB/CAR/PDMS), was used to collect aroma volatiles from treacle headspace bottles. The fibre was exposed at a constant depth into the headspace of the sample bottle at 35 °C for 20 min.

2.3. Desorption and analysis of the volatile compounds

The SPME fiber was placed immediately into the injection port of the GC-MS system (HP G1800A GCD system, Agilent, Waldbronn, Germany). The fibre was left in the injection port for 10 min. Injector temperature was 270 °C. The injector port was equipped with a glass liner especially designed for SPME measurements (0.75 mm i.d. splitless glass liner, Supelco). Injection took place in splitless mode, and the split valve was opened after 2 minutes. The desorbed aroma volatiles were separated on an HP5 (cross-linked 5% phenyl methyl siloxane) capillary column (30 m × 0.25 mm i.d.; film thickness 1 μm). Helium with a purity of 99.999% (Air Liquide, Austria) was used as carrier gas. Column head pressure was 0.16 bar at −10 ° C and constant flow was maintained for the whole run. The initial temperature was −10 °C (hold time 1 min), with a temperature increase of 12 °C/min, to a final temperature of 280 °C (hold time 1 min). Temperatures below 45 °C were controlled by blowing liquid nitrogen into the GC oven. Detector temperature was 280 °C. Electron impact ionisation was used (70 eV). Data were acquired in scan mode, scanning a mass range from 20 amu to 350 amu.

2.4. Determination of HMF

2.4.1. Sample preparation

One gram of treacle was dissolved in 100 ml water and centrifuged at 14 000 rpm for 10 min (Eppendorf 5804 R, Hamburg, Germany). This solution was used for HPLC analysis. Reversed phase HPLC was carried out on an HP 1100 (Agilent, Waldbronn, Germany), equipped with a quaternary pump, vacuum degasser, autosampler, and variable wavelength detector. The column was a Lichrocart Purospher Star 100 (55 × 2 mm, 3 μm). The elution was done isocratically with a mixture of methanol (5%) and water (95%) using a flow rate of 0.3 ml/min and an injection volume of 3 μl. HMF was detected at its absorption maximum of 280 nm. The limit of detection was 7 ng/ml. The recovery was 89% as determined by standard addition. The standard deviation at 200 ng/ml was 1.4%.

HMF was quantified using the external standard method with five calibration levels of HMF, spanning the expected concentration range.

2.5. Determination of carbohydrates

One gram of treacle sample was dissolved in 200 ml water and centrifuged at 14 000 rpm for 10 min (Eppendorf 5804 R). This solution was used for HPLC analysis, which was carried out using an HP 1100 (Agilent) equipped with a quaternary pump, vacuum degasser, autosampler, and refractive index detector. The column was a fast acid column (BioRad) with 5 mM sulfuric acid in water as eluant using a flow rate of 1 ml/min and an injection volume of 10 μl.

3. Results and discussion

Treacle production is considered to be a multi-stage evaporation process for sugar cane juice using heat. During this process different aliphatic aldehydes, ketones, furan derivatives and lower fatty acids are formed as a result of heat-induced non-enzymatic browning reactions (Vernin & Vernin, 1982). Different companies are involved in the production of treacle and each brand name has its own taste and flavour depending mainly on the sugars content and the processing parameters during production, including time and temperature of water evaporation from the sugar cane juice. Table 1 shows the sugar content and insoluble matter of the three investigated commercial treacle samples.

Table 2 shows the variation in the aroma composition of the three treacle samples. From the table it is evident that aliphatic acids (particularly acetic acid) occurred at relatively high concentrations in the three treacle samples, with the highest levels in sample B (40.1%), while samples A (22.5%) and C (26.3%) have similar concentrations. The presence of acetic acid in treacle volatiles is common and expected. Acetic acid was previously reported as a major volatile constituent in the aroma of brown sugar obtained

Table 1
Sugar and insoluble matter content of three different commercial treacle samples

Sample	Sucrose (g/kg)	Glucose (g/kg)	Fructose (g/kg)	Insoluble matter (%)
A	404	137	138	1.0
B	329	186	170	0.69
C	417	156	149	0.72

Table 2
Volatile constituents of three different commercial treacle samples as determined by HS-SPME

Compound	RT(min)	RI[a]	Sample A		Sample B		Sample C	
			Area (%)[b]	RSD (%)[g]	Area (%)[b]	RSD (%)[g]	Area (%) [b]	RSD (%)[g]
Ethanol[c]	4.19	<600	0.4	6.1	1.0	3.1	31.7	3.3
Acetone[c]	4.83	<600	0.7	4.2	0.2	2.7	0.2	3.5
Dimethylsulfide[c]	5.37	<600	12.8	5.1	8.1	5.3	1.9	12.4
2-Methylpropanal[d]	6.19	552	4.7	0.6	2.3	1.1	0.2	5.7
2-Butanone[d]	6.96	597	0.6	1.7	0.2	0.9	0.2	6.9
2-Methyl-3-buten-2-ol[d]	7.27	620	0.7	6.1	nd[f]		nd[f]	
2-Methyl-1-propanol[d]	7.61	626	nd[f]		nd[f]		2.5	11.1
Acetic acid[e]	7.65	–	22.5	2.1	40.1	1.7	26.3	0.5
3-Methylbutanal[d]	8.13	648	6.4	0.4	5.3	1.9	0.5	6.3
2-Methylbutanal[d]	8.33	658	12.7	1.8	6.3	1.7	0.4	7.8
1-Hydroxy-2-propanone[c]	8.47	–	4.4	5.4	2.6	4.0	3.1	2.5
Propanoic acid[e]	8.8	–	1.9	1.9	1.4	0.3	0.9	6.5
3-Hydroxy-2-butanone[d]	9.26	707	0.9	0.0	0.7	0.9	0.9	5.1
3-Methyl-1-butanol[d]	9.63	730	nd[f]		nd[f]		5.8	3.5
2-Methyl-1-butanol[d]	9.71	733	nd[f]		nd[f]		7.6	0.4
Butanoic acid[e]	10.38	–	1.3	7.0	1.1	1.3	2.8	1.5
2,3-Butanediol[c]	10.43	–	0.0		1.0	2.8	0.0	
Dihydro-2-methyl-3(2H)-furanone[c]	10.95	–	8.2	1.4	4.7	2.1	4.2	0.2
2-Furfural[d]	11.53	830	5.7	2.5	10.8	1.7	0.4	1.8
2-Furfuryl alcohol[d]	11.75	852	1.8	4.7	0.9	14.3	2.4	0.2
2-Acetylfuran[d]	12.73	910	3.5	0.9	2.4	1.3	2.4	2.3
5-Methyl-2-furancarboxaldehyde[d]	13.58	962	1.2	1.7	1.3	4.8	nd[f]	
Aliphatic acids			25.7		42.6		30.0	
Aliphatic alcohols			1.1		2.0		47.6	
Aliphatic aldehydes			23.8		13.9		1.1	
Aliphatic ketones			6.9		3.7		4.4	
Sulfur compounds			12.8		8.1		1.9	
Furan derivatives			20.4		20.1		9.4	
Amount of the total area (%)			90.7		90.4		94.4	

[a] The retention indices given (on an HPS column) are those, where the measured values are in good accordance with the tabulated values in a retention index database.
[b] The given values are the arithmetic means from duplicate analysis. Differences between the single measurements were not higher than 10% for the whole procedure. Areas were normalized to a sample weight of exactly 200 mg.
[c] Tentatively identified; the identification was based on the mass spectra and comparison of the spectra with those from a mass spectra library (Wiley 275). The accordance of the spectra with the mass spectra from the MS database was very high (>90%).
[d] The identification was based on the mass spectra and comparison of the spectra with those from an MS library (Wiley 275). In addition the obtained retention indices were compared with those from a retention index database.
[e] The organic acids acetic acid, propionic acid and butyric acid were identified based on their retention behaviour, the typical peak shape of the polar compounds on the non-polar stationary phase of the analytical column and the mass spectra in comparison with those from the MS database (Wiley 275). Due to the strong fronting of the peak, no retention index could be calculated.
[f] Not detected.
[g] Relative standard deviation.

from cane (Godshall & DeLucca, 1984). It was known a long time ago that the origin of acetic acid is attributed to the thermal degradation of sugars during the Maillard reaction (Hodge, 1953). Davidek, Clety, Aubin, and Blank (2002) indicate that acetic acid is formed in high levels from free and protein-bound Amadori compounds, and depending on the reaction conditions, the yield of acetic acid may reach up to 60 mol%. In a glucose-based Maillard reaction, the β-dicarbonyl cleavage of l-deoxyhexo-2,4-diulose, (Maillard key intermediate) is proposed to be the major pathway leading to the formation of acetic acid (Davidek, Devaud, Robert, & Blank, 2005).

Microbiological fermentation due to the presence of high sugar content, as well as other nutrients in treacle, should also be taken into consideration as a second possible source of acetic acid in treacle samples (Godshall & DeLucca, 1984). Besides acetic acid, propanoic and butyric acids are found to a lesser extent in all treacle samples.

Trained panelists in our lab could not perceive acetic acid in the three treacle samples (unpublished data), mainly due to the interactions between acetic acid and the other constituents of treacle, These interactions modify the overall release and perception of acetic acid.

Aliphatic alcohols represent the major volatile constituents in treacle sample C only, while samples A and B contain a modest amount of alcohols. Ethanol is the main aliphatic alcohol in sample C, which probably indicates a high fermentation activity in this sample. The extent of fermentation in treacle can be judged by visual examination of the foaming layer (CO_2 bubbles) on the treacle surface in

the jar. In addition to ethanol, some fusel alcohols were only detected in sample C. These alcohols included 3-methyl-1-butanol (isoamyl alcohol) and 2-methyl-1-butanol (active amyl alcohol). These alcohols are supposed to originate from the microbial transformation of the amino acids leucine and isoleucine, respectively (Elrich, 1907; Neubauer & Fromherz, 1911). These amino acids, in turn, are formed by the hydrolysis of proteins present in the cane juice or the protein of the microorganism cells themselves (Elrich, 1907). The reason why such fusel alcohols did not exist in samples A and B could be attributed to the lack of the proper microbial flora in these samples.

Dimethyl sulfide is found in the volatiles of the three treacle samples. This compound is a typical constituent of garlic and onion essential oils. Its presence could be objectionable in some food products due to its strong sulfurous aroma. However, its presence in very low concentrations has been reported to have a beneficial effect on the aroma of some beverages like wines (Simpson, 1979). Dimethyl sulfide was isolated and identified for the first time from the volatiles of molasses, and was described as an important compound in molasses aroma (Godshall, Roberts, & Legendre, 1980). The origin of dimethyl sulfide could be attributed to the microbial activity of yeast and bacteria (De Mora, Eschenbruch, Knowles, & Spedding, 1986).

Furan derivatives reported in Table 2 are typical products of non-enzymatic browning. They are responsible for the caramel, burnt-sugar aroma characterising the flavour of treacle, while the other reported volatile aldehydes and ketones also contribute to the full treacle flavour impact.

HMF is a major intermediate of the Maillard reaction, and also as a dehydration product of hexoses (glucose and fructose) in the absence of amino acids (Anam & Dart, 1995). However, the easy formation of HMF from fructose or the fructose monomer of sucrose is most common (Antal, Mok, & Richards, 1990). HMF can be formed at temperatures as low as 50 °C, so it could be taken as an indicator of heat stress to foods during processing.

HS-SPME could not isolate HMF, probably due to its low abundance in the headspace of the sample and/or the poor affinity between that component and the DVB/CAR/PDMS fibre material. Thus, identification and quantitation of HMF was performed using HPLC, which is the standard method of analysis. The results are shown in Table 3.

The amount of HMF varies among the three treacle samples from 66 mg/kg to 179 mg/kg. No limit was set by the Codex Alimentarius Commission Standards for HMF in treacle (personal communication 2006). However, the acceptable limits of HMF in bee honey, for example, lie between 0 mg/kg and 40 mg/kg before storage for six months (Gidamis, Chove, Shayo, Nnko, & Bangu, 2004). However, honey producers from hot countries are petitioning to the standards commission to raise the acceptable limits to 80 mg/kg. Very high levels of HMF have been reported in some food products, such as dried pears (3.5 g/kg), caramel products (9.5 g/kg) (Bachmann, Meier, & Känzig, 1997), instant coffee powder (6.2 g/kg) and coffee substitutes (13.9 g/kg) (Schultheiss, Jensen, & Galensa, 1999).

Table 3
Content of 5-hydroxymethylfurfural (HMF) in three different commercial treacle samples

Sample	HMF content (mg/kg sample)	SD[a] (mg/kg)
A	66.1	0.9
B	179.0	2.5
C	92.4	1.3

[a] Standard deviation.

HMF formation in treacle or any other heated food product is unavoidable. In order to reduce its quantities in treacle the processor should mainly control the temperature and time of the evaporation process. This could be achieved by carrying out the process under reduced pressure. Also, reduction of the time period between cutting the cane stalks from the field and juicing before heat processing is essential. If this period increases, invertase enzyme present in cane stalks will increase the conversion of sucrose into reducing sugars, glucose and fructose, thus increasing the potential for HMF formation during heat processing.

In conclusion, we report here, for the first time, the identification of the major aroma components in treacle, in addition to the quantitation of HMF. Headspace-solid-phase micro extraction with a DVB/CAR/PDMS fibre is an effective and fast technique for the comparison of different treacle samples. Measurement of ethanol and fusel alcohols, as well as acetic acid, will be of interest in quality control laboratories measuring the extent of fermentation in different treacle samples. The reported aroma profile of treacle can help the food processor/technologist to determine new food applications for treacle, as a natural flavouring agent, responsible for caramel, sweet, burnt-sugar aroma. HMF quantitation will guarantee safety, taking into consideration that the concentration of this compound will be diluted several-fold in the final product, when treacle is mixed with the other ingredients.

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