

Aroma Compounds in the Production of Liquid Beet Sugar

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Samples of in-process liquid beet sugar were collected from three different parts of a beet sugar factory and a refinery. The samples were analyzed with respect to aroma compounds by means of both liquid–liquid extraction and gas-phase (headspace) extraction followed by gas chromatography–olfactometry (GC–O) and GC–mass spectrometry (GC–MS). The aromas of the eluted compounds were evaluated qualitatively and quantitatively for the different samples. In general, earthy and sour aromas were often present in the raw juice sample, whereas caramel aromas were mainly present in the samples taken further downstream in the process. For fruity, floral, and solvent-like aromas, different parallel trends were noted. Some aromas were present only at the beginning of the process, whereas others developed toward the end of the process.

Keywords: *Liquid beet sugar; aroma; flavor; gas chromatography–olfactometry; GC–MS*

INTRODUCTION

The use of liquid sugar (sucrose) rather than crystal sugar has proved successful in many industrial applications, as this product is easier to handle and its quality is more easily maintained and modified according to the customers' needs than is crystal sugar. Liquid sugar can be manufactured by dissolving white crystal sugar in water or by using process syrups from a sugar refinery. In the latter process, less total crystallization is needed to obtain the final product. This approach makes the overall process of sugar manufacturing more efficient because the total loss of sugar during processing is reduced. However, the requirements for purity differ between applications, and because the aroma and flavor intensity of the less crystallized sugar are higher, unwanted aromas and flavors may be experienced in some applications. It is, therefore, very important to be able to maintain the aroma and flavor at levels adjusted to the different requirements of various food applications.

There are many different causes for the aroma and flavor in different production streams in a sugar refinery. The components found in beet sugar products can be divided into the following groups (1): metabolites of the beet itself, compounds formed in the sugar beet as a consequence of microbial activity in the soil and of compounds that are sorbed by the beet during growth, and finally, compounds formed during storage or in the process of sugar manufacturing, the latter of which are often referred to as "sugar degradation products". Typical process aromas may come from nonenzymic browning (Maillard reactions and caramelization) or from ion exchange and adsorbent resins that are used for purification of liquid sugar products (2).

There is obviously a large difference between the composition of volatile compounds at the beginning of the process of sugar manufacturing and those from toward the end. This is because most of the compounds originating from the sugar beet are removed in the

process. On the other hand, new compounds appear through the mechanisms discussed above (2).

One way of characterizing and quantifying the aroma intensity in a sample is by using GC–olfactometry (GC–O). In this technique, the separated compounds at the effluent from a GC column are evaluated qualitatively and quantitatively, one by one, by human assessors. There are different approaches to GC–O, one of which is denoted aroma extract dilution analysis (AEDA) and is a tool to screen the most aroma-active volatile compounds in, for instance, a food extract (3). AEDA is often referred to as a dilution technique, which means that a series of dilutions of the sample are prepared and analyzed by GC–O. Each successive dilution is sniffed until no significant aroma is perceived. The number of dilutions necessary to eliminate the presence of an aroma is used to estimate the aroma potency of compounds giving rise to chromatographic peaks (4).

With another dilution technique, the so-called CharmAnalysis, the aroma intensity for eluting compounds is marked and graphed. The resulting graph resembles a chromatogram and consists of peaks that can be integrated to provide peak areas (charm values). The greater the charm value the more potent the aroma contribution of the peak (5).

Yet another approach to GC–O is the so-called Osme technique. Osme is a quantitative method used to measure the perceived aroma intensity of a compound eluting from a GC–olfactometer. The assessor rates the intensity of the compound's aroma by using a time–intensity device, thus providing an aroma peak (similar to the peak on a gas chromatogram). At the same time, verbal descriptions of the aroma peak are recorded. This method is different from the CharmAnalysis and AEDA methods in that Osme is not based on aroma detection thresholds (6).

The objective of this investigation was to characterize the compounds responsible for aroma in selected products in the process of sugar manufacture. From earlier investigations, the results from liquid–liquid extraction and headspace sampling of identical samples differed with regard to the compounds detected (2). This discrepancy in results between methods may give an

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unclear picture of the compounds that actually are present and may give rise to aromas and flavors. In this study, three different samples from the liquid-sugar manufacturing process were extracted both with solvent and with gas (headspace sampling) and thereafter analyzed with GC–O in order to obtain a more complete picture of the true aroma profile of the different samples, and to determine where various aromas were the most intense and where they disappeared altogether. A device was developed making possible the use of both peak integrations and peak heights as measures of aroma intensity. The samples were also analyzed with liquid–liquid and headspace GC–MS in order to elucidate which compounds were responsible for the perceived aromas.

Analyzing both liquid–liquid extracts and trapped headspace volatiles with GC–O in combination with GC–MS may give information about which compounds in the sample give rise to aromas and flavors, and also, which compounds would possibly do so if their concentrations were higher. It should be emphasized, however, that dual extraction was not performed primarily in order to study the extraction methods, but to get closer to forming a true picture of the volatile compounds giving rise to aromas and flavors in liquid sugar, and of their formation and decrease in concentration due to purification.

MATERIALS AND METHODS

Safety Note. Explosive peroxides may form in diethyl ether, especially if evaporated to dryness. A peroxide test must therefore be performed prior to any work with this solvent, as was performed in this study.

Samples. In the sugar manufacturing process, the cleaned and sliced beets are extracted with hot water (70 °C). The resulting solution is referred to as raw juice. A raw juice sample (sample A) was taken from Danisco Sugar AB's beet sugar factory in Jordberga, Sweden, during the beet campaign of 1999. A refinery syrup (sample B) taken after the boiling pans, containing a mix of different syrups and sugars from the different pans and intended for liquid sugar production, was taken from the Danisco Sugar Arlöv refinery, Sweden. A final liquid sugar for industrial use (sample C) was likewise taken from the Danisco Sugar Arlöv refinery. A schematic diagram of the beet sugar factory and refinery processes with sampling points is given in Figure 1. All samples were not from the same bulk beet source because the raw sugar and the refined products are produced at two different locations.

Sugar Analyses. The investigated samples were analyzed according to the International Commission for the Uniform Methods of Sugar Analysis (ICUMSA) methods regarding dry substance content, invert sugar content, pH, color, and ash content. The invert sugar content and the ash content are given as the ratio between the amount of the respective parameter and the dry substance content on a weight basis.

Sensory Analysis of Total Aroma. Samples B and C from the refinery process were evaluated by sensory analysis to form an idea about the total aroma in the samples. Eight assessors (6 women, 2 men, all experienced) were used. The samples, presented in protective aroma glasses and under red light to prevent influence of color, were evaluated on a scale of 0–9 on the basis of the attribute "total aroma". Sample A was not evaluated on the basis of "total aroma" because the aroma of raw juice is very different from that of refinery syrup (sample B) and liquid sugar (sample C), so a comparison would have been misleading.

Headspace Sampling. Volatile compounds from the samples A, B, and C were adsorbed on Tenax TA (60–80 mesh) by the method described by Hall et al. (7). Each sample (100 g in a 500-mL flask) was allowed to equilibrate for 30 min at 40 °C

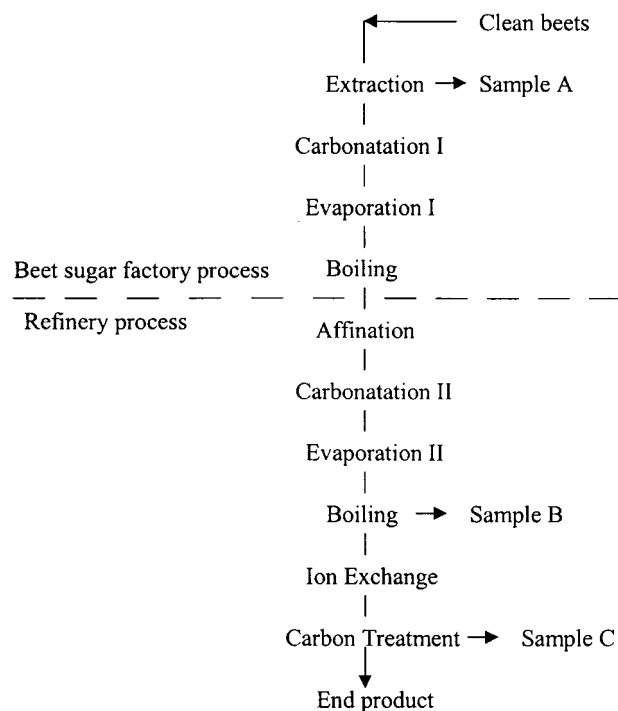


Figure 1. Schematic diagram of the process of raw liquid sugar production and subsequent refining. The samples collected and analyzed are labeled A–C.

with stirring. Then 5 L of helium was led through the flask and allowed to pass the adsorbent material at a rate of 40 mL/min.

Liquid–Liquid Extraction. The samples A, B, and C were extracted by means of liquid–liquid extraction, for which 100 g of sample was shaken with 20 mL of diethyl ether with an added internal standard (hexyl acetate, 1.5 µg/mL) in a separatory funnel, and the ether phase was recovered. The extraction, performed at room temperature, was repeated twice and the three portions of ether were united. The ether extract was dried on dry sodium sulfate prior to evaporation. The evaporation was done at 35 °C. A 15-cm-long column was connected to the evaporation flask (Vigreux flask). The final volume after evaporation was approximately 200 µL.

Gas Chromatography–Olfactometry. The gas chromatograph (Varian 3200, Walnut Creek, CA) used for GC–O was equipped with both an injector for liquid extracts and an inlet from an automatic thermal desorption system, ATD400 (Perkin-Elmer, Norwalk, CT), the latter permitting desorbed volatile compounds from gas-phase extraction to be analyzed.

The volatile compounds adsorbed on Tenax TA were thermally desorbed (5 min at 250 °C) and subsequently injected into the gas chromatograph. A volume of 2 µL of diethyl ether extract was injected to the chromatograph by means of a syringe.

The separation was performed with the help of a 30 m × 0.32 mm capillary column with a 1.0-µm-thick film of DB-1 (J&W Scientific Inc., Folsom, CA). The initial temperature of the GC oven was 35 °C and the temperature was increased by 4 °C per minute until a final temperature of 220 °C was reached. The oven was kept at the final temperature for another 20 min. Identical temperature programs were used for the volatiles extracted by liquid–liquid extraction and gas-phase extraction. The effluent of the capillary column was split 1:1 between a flame ionization detector (FID) and a sniffer-port, in the latter of which the column effluent (1.5 mL/min) was mixed with humidified air (100 mL/min) in order to facilitate the sensory evaluation. Four assessors (1 woman, 3 men, all experienced) evaluated the effluent of the capillary column in the following way: the first 20-min period of chromatographic separation was evaluated by assessor 1, the following 20-min period was evaluated by assessor 2, and the

last 20-min period was evaluated by assessor 1. Aromas were rarely perceived in the last 20-min period of the chromatogram, but the chromatograms from all runs were evaluated in this way. In the second chromatographic run, the order of assessors 1 and 2 was permuted so that both assessors evaluated one whole chromatogram. The process was repeated for assessors 3 and 4. The three samples, extracted by both liquid-liquid extraction and gas-phase extraction, were evaluated in this way, giving a total of 24 analyses. Prior to the assessment, the assessors were allowed to consult a word list containing typical aroma words developed from earlier investigations (8). The intensity of a perceived aroma was indicated by each assessor sliding a linear potentiometer from left (no aroma) to right. The assessors did not see the FID response to avoid influence from the latter. The signal from the circuitry was split, so that the signal could be registered by a laboratory data system (HP 3550, Hewlett-Packard, Palo Alto, CA) and a printer. A secretary stationed at that printer noted the aroma attributes for each peak as they were mentioned by the assessor.

Data Handling (Gas Chromatography-Olfactometry). The intensities of perceived aromas could be expressed as peak heights, measured on the recorder paper, or as peak areas, as registered by the laboratory data system. The peak areas had to be converted into relative values; that is, every peak area for one assessor was divided by the total area for that assessor prior to formation of mean values, because the variation between assessors was very large and forming a mean value of the untreated data would have given a distorted result. To decide whether to use peak height or peak area as a response for the sensory intensity the variability between peak heights and peak areas for different assessors was estimated by calculating and comparing the relative standard deviations (RSDs).

Only aromas perceived by three or four of the four assessors were treated; the others were considered not quantifiable. Mean values of the raw measurements were calculated. The intensity measurements (mean values of peak heights) were then converted into a discrete sensory scale of 0-5, where 0 corresponds to no aroma and 5 corresponds to an intense aroma. The peaks from the FID signal of the internal standard were quantified. These quantifications from the different chromatographic runs were thereafter compared to ensure that differences in evaporation of the solvent were not significant, which would have distorted the results.

Gas Chromatography-Mass Spectrometry. Gas chromatography-mass spectrometry was performed on both headspace volatiles desorbed from Tenax TA and diethyl ether extracts. The settings of the gas chromatograph were the same as those for the GC-O runs. The carrier-gas flow was adjusted so that the retention time for the internal standard (liquid-liquid extraction) and hexanal and nonane from a calibration solution (gas-phase extraction) was identical to the one for the respective GC-O runs. The liquid-liquid extracts were injected to a Thermoquest trace GC 2000 (Thermoquest, San José, CA) by means of a CTCA200S autosampler (CTC). To the gas chromatograph was coupled an Automass Solo mass spectrometer (Thermoquest). The headspace volatiles were thermally desorbed and injected to a Thermoquest trace GC 2000 (Thermoquest) using an ATD400 (Perkin-Elmer). An Inco 50 mass spectrometer (Finnigan, San José, CA) was coupled to the gas chromatograph.

RESULTS AND DISCUSSION

Samples. The samples A, B, and C each represent three different stages in the process of liquid sugar manufacturing. Typically, sample A would be expected to contain components of the sugar beets; sample B would be expected to contain a mixture of compounds from the sugar beets and thermally induced compounds (sugar degradation products); and sample C should preferably be devoid of compounds from the sugar beets,

Table 1. Sugar Analyses (ICUMSA methods)

sample	RDS% ^a	color ICU ₄₂₀	pH	ash content
A	16.0	2300	4.1	0.4
B	62.5	1350	7.6	0.43
C	62.8	32	7.0	0.011

^a RDS%, refractometric dry substance.

and thermally induced compounds should be heavily reduced as compared with sample B. Earlier investigations showed that this is not always the case (2). Sample C may also include compounds released from the different purifying steps taken between samples B and C.

Sugar Analysis. The results from the sugar analyses are shown in Table 1. The refractometric dry substance (RDS%), a measure of the sugar content, in sample A was approximately one-fourth that of sample B and C. There was also a great difference in color between the three samples, especially where color was related to RDS%. The pH was much lower in sample A than in samples B and C, which was to be expected because two carbonatation steps involving the addition of lime, which raises pH, were performed between sample A and samples B and C. The ash content, related to RDS%, was higher for samples A and B than for sample C, probably due to the removal of ash in ion exchangers prior to the point of collection of sample C.

Sensory Evaluation of Total Aroma. In the sensory evaluation of samples B and C (total aroma), as rated on a scale of 0-9, sample B (7.4 ± 1.3) exhibited a stronger aroma than did sample C (4.1 ± 1.1). Means and standard deviations ($n = 8$) are given. All assessors rated sample B at least two units higher than sample C.

Gas Chromatography-Olfactometry. Typical RSDs between assessors for the peak heights were 5.8-41% (mean value 24%). The corresponding values for peak areas were 15-85% (mean value 52%). Therefore, peak heights, rather than peak areas, were used in the evaluation. It has to be mentioned, however, that the general trends observed for peak heights, with some exceptions, are valid for peak areas as well. The aromas will, in the following, be referred to by their retention time (in minutes).

Gas-Phase Extraction. In Table 2, the perceived aromas, the aroma intensities for samples A, B, and C, the aroma groups as defined below, and the identified compounds are summarized. In Table 2, the number of assessors who perceived an aroma is given in brackets after the intensity value. Aromas perceived by only one or two of the assessors are referred to as not quantifiable (Nq), and the number of assessors who actually perceived the aroma is given in brackets. If an aroma was noted by fewer than three assessors in all three samples the aroma in question was removed from the table. The number of perceived aromas was 31 in sample A, 19 in sample B, and 14 in sample C. Many different aromas were perceived, and in order to be able to draw comprehensible conclusions about the trends of the perceived aromas similar aromas were evaluated together. The different aromas encountered were classified into one of the following groups: sour, manure-like (group I); earthy, moldy (group II); caramel-like (group III); floral, green, and solvent-like (group IV); and ester-like (solvent, fruity) (group V). Of these five groups, groups I-III came up naturally whereas the groups IV and V were more difficult to define.

Table 2. Results of Headspace-GC-O

retention time (min)	perceived aroma	aroma group ^a	sample A	sample B	sample C	identified compound
3	cabbage, musty, boiled milk, solvent	IV	Nq (1) ^{b,c}	4 (4)	Nq (2)	
4.3	butterscotch, artificial, vanilla	III	4 (4)	Nq (2)	4 (4)	butanone
5	salubrin	V	3 (3)	Nq (0)	Nq (0)	ethyl acetate
5.4	salubrin, nutty	V	4 (4)	Nq (0)	Nq (0)	
5.9	nutty, solvent, raisins	IV	5 (3)	Nq (2)	Nq (2)	2-methylbutanal
6.8	boiled milk, butterscotch	III	Nq (0)	Nq (2)	4 (3)	3-hydroxy-2-butanone
7.8	butterscotch, salubrin	V	5 (3)	Nq (1)	Nq (0)	ethylpropanoate
8.4	pungent, solvent, glue	IV	Nq (2)	4 (4)	Nq (0)	3-methylbutanol
8.5	acrid, rotten, onion	I	Nq (0)	Nq (1)	4 (3)	dimethyl disulfide
9.4	bitter, stuffy, sour	I	4 (4)	Nq (0)	Nq (0)	
9.5	bitter, solvent, plastic	V	4 (4)	Nq (2)	3 (3)	2-methylpropyl acetate
10.4	green, grass	IV	3 (4)	4 (3)	4 (3)	hexanal
10.8	fermented, candy, apple juice	V	4 (4)	Nq (0)	Nq (0)	ethylbutanoate
12	manure, rotten eggs	I	4 (4)	5 (3)	4 (4)	
12.3	dihydrogen sulfide	I	4 (3)	Nq (0)	Nq (0)	
13.3	boiled milk, fatty	III	4 (3)	Nq (0)	Nq (0)	
13.7	nail polish, sweet solvent	V	4 (4)	Nq (0)	Nq (0)	2-methylbutyl acetate
14.6	glue, solvent, ethereal	IV	3 (3)	4 (4)	4 (4)	ethylpentanoate
15.0	burned almonds, toska, nutella	III	4 (3)	4 (4)	5 (4)	2,5-dimethylpyrazine
15.4	peanut butter, burnt	III	Nq (1)	Nq (2)	5 (3)	
17.1	asparagus, humid/old clothes, sulfur	I	Nq (2)	4 (3)	Nq (0)	
17.5	mushroom, humus	II	3 (4)	3 (3)	3 (3)	
18	floral, fruity, ethereal	IV	Nq (1)	4 (4)	Nq (2)	
18.5	floral, plant, ethereal	IV	Nq (2)	Nq (0)	4 (4)	methyl-2-methylpentanoate
19.2	caramel	III	Nq (0)	3 (3)	Nq (0)	
19.3	sweet, melon	V	3 (3)	Nq (0)	Nq (0)	
19.7	floral, pelargonium,	IV	4 (4)	5 (3)	Nq (2)	
20.6	burnt, caramel, nutty	III	Nq (0)	3 (4)	Nq (0)	3-ethyl-2,5-dimethylpyrazine
20.7	floral, smoke, crushed insects	IV	4 (4)	Nq (0)	Nq (0)	
21.3	chocolate, burnt	III	Nq (0)	5 (3)	Nq (2)	
21.4	burned circuitry/burned wood	II	4 (4)	Nq (0)	Nq (0)	
21.6	mushroom, slightly green	IV	Nq (0)	Nq (0)	4 (4)	
21.8	solvent, ethereal, green	IV	3 (4)	4 (4)	Nq (0)	2,5-diethylpyrazine
22.4	mushroom, celery, green	IV	4 (4)	3 (3)	Nq (0)	
22.8	sweet floral, ink, candy	IV	3 (4)	Nq (2)	Nq (2)	nonanal
24.5	floral, nutty, earthy	IV	Nq (2)	4 (3)	4 (4)	
24.9	old plastic, tennis court, rubber	II	3 (3)	Nq (0)	Nq (0)	
25.3	paint, rubber, burnt, plastic	IV	4 (4)	Nq (2)	Nq (2)	
25.6	hospital, broken stems, solvent	IV	4 (3)	Nq (2)	Nq (2)	
26.1	earthy, mold, plant, warm circuitry	II	Nq (2)	3 (3)	3 (3)	nonanal
29.5	plastic, floral, sweet, strawberry	V	Nq (0)	4 (3)	Nq (1)	
31.0	earthy, moldy, rubber shoes	II	3 (3)	Nq (0)	Nq (2)	
31.4	sweet, plastic, candy	V	3 (4)	Nq (0)	Nq (0)	
32.7	earthy, moldy, chalk	II	4 (4)	Nq (0)	Nq (1)	
33.1	butterscotch, chocolate, slightly floral	III	3 (3)	3 (3)	Nq (0)	
34	earthy, moldy, vanilla, bakery	II	4 (3)	Nq (0)	Nq (1)	

^a For an explanation of the aroma groups, see the Gas-Phase Extraction section. ^b The number of assessors who perceived the aroma is given in brackets. ^c Nq = not quantifiable.

For sour, manure-like aromas (I), it was difficult to find obvious patterns and draw any clear-cut conclusions. Two aromas (9.4 and 12.3) were present only in sample A, one (8.5, identified as dimethyl disulfide) was present only in sample C, one (17.1) was present only in sample B, and one (12.0) was present at high intensity in all three samples. Dimethyl disulfide has been investigated previously in relation to sugar refining (2), but in that study it was present only in the raw juice (i.e., sample A). The sour, manure-like aromas are often caused by carboxylic acids that sometimes are difficult to extract with gas-phase extraction. Volatile carboxylic acids have been previously claimed to be responsible for aromas and flavors in beet sugar (1). Marsili et al. (9) have identified acetic acid, propanoic acid, butyric acid, and isovaleric acid in crystal sugar using purge and trap techniques.

In general, the earthy aromas (II) are the most intense in raw juice (i.e., sample A). Exceptions to this are the compound eluted after 17.5 min, which exhibits aromas at high intensity in raw juice as well as in

refinery syrup and liquid sugar, and the compound eluted after 26.1 min, which in this study gave off intense aromas in samples B and C. The only earthy aroma for which the corresponding compound was identified was eluted at 26.1 and the compound was nonanol. Nonanol has been reported to have a rose-orange aroma, but the aroma characteristics are influenced by concentration (10).

The general pattern for the caramel aromas (III), which group comprises aromas such as burnt sugar, butterscotch, burned almonds, and nut, is that they are absent, or present at a low level, in the raw juice (sample A) and increase after the heat treatment in later process steps. However, there are important exceptions to this. The aroma 4.3 was present only in samples A and C, 13.3 was present only in sample A, 15.0 was present in all three samples, and 33.1 was present in samples A and B. It would have been anticipated that the aroma intensity would decrease in the last sample because this sample is subjected to many purifying operations. However, some caramel-like

Table 3. Results of Liquid-Liquid Extraction-GC-O

retention time (min)	perceived aroma	aroma group ^a	sample A	sample B	sample C	identified compound
5.4	ether		5 (4) ^b	5 (4)	5 (4)	diethyl ether, solvent
8.6	butterscotch, sweet	III	Nq (0) ^c	2 (3)	Nq (0)	
9.5	sour, tennis shoes	I	4 (3)	Nq (0)	Nq (1)	2-methylbutanoic acid
12.2	manure, tennis shoes, pungent	I	4 (4)	Nq (0)	Nq (0)	hexanoic acid
15.0	manure, tennis shoes, pungent	I	5 (3)	Nq (0)	Nq (0)	2-methyl-hexanoic acid
16.5	manure, tennis shoes, pungent	I	3 (4)	Nq (0)	Nq (1)	octanoic acid
17.5	rubber, boiled milk, bakery, musty	III	4 (3)	Nq (1)	Nq (1)	
18.4	boiled potato, burnt	III	Nq (1)	Nq (2)	5 (3)	acid
19.2	floral, solvent, ethereal	IV	Nq (0)	Nq (2)	4 (4)	2-ethyl-5,6-dimethylpyrazine
19.6	burnt, caramel, boiled potato	III	Nq (1)	5 (4)	4 (3)	hexyl acetate internal standard
23.4	solvent, ether, pungent	IV	Nq (1)	Nq (0)	4 (4)	
24.0	nutty, solvent, floral, burnt sugar	III	Nq (0)	3 (3)	4 (4)	
24.4	burnt sugar, bakery, sweet	III	Nq (0)	Nq (0)	3 (4)	
24.9	floral, grass, solvent	II	4 (3)	Nq (0)	Nq (0)	alcohol
25.5	caramel, boiled milk, butterscotch	III	Nq (0)	4 (4)	4 (4)	
26.0	wet cardboard, old football	II	3 (4)	Nq (0)	Nq (0)	acid
26.2	sour, burnt cellulose (caramel)	III	Nq (1)	4 (4)	4 (3)	
26.4	ethereal, floral, smoke	IV	Nq (0)	Nq (1)	5 (4)	
26.8	ether, caramel, doctor's consulting room	III	Nq (0)	Nq (2)	5 (3)	
27.3	earthy	II	4 (4)	Nq (1)	Nq (0)	
27.9	yeast, floral, ink	IV	3 (3)	Nq (2)	Nq (2)	
28.7	burnt, polymers, sweet, vanilla	III	Nq (2)	4 (3)	Nq (0)	
29.5	earthy, green, musty	II	4 (3)	Nq (2)	Nq (0)	
29.8	plastic, solvent, leather, sweat	IV	Nq (2)	Nq (2)	3 (4)	
30.6	earthy, potato, plant	II	4 (3)	Nq (1)	Nq (0)	geosmin (fragment 112)
31.8	almonds, nauseating	III	4 (4)	Nq (0)	Nq (0)	
32.7	manure, rubber, sour	I	4 (3)	Nq (0)	Nq (0)	decanoic acid
33.0	almonds, caramel, burnt	III	Nq (0)	Nq (2)	3 (4)	
34.5	spicy, floral, sweet	IV	4 (3)	Nq (2)	Nq (0)	
35.5	floral, spicy, dentist's consulting room, fireworks	IV	Nq (2)	5 (3)	4 (4)	
36.9	beeswax, sweet, incense	IV	3 (3)	Nq (0)	Nq (2)	
38.0	vanilla, sweet, almonds	III	Nq (0)	4 (4)	4 (4)	
38.6	vanilla, oak tree, wet rag	III	Nq (1)	3 (3)	Nq (2)	

^a For an explanation of the aroma groups, see the Gas-Phase Extraction section. ^b The number of assessors who perceived the aroma is given in brackets. ^c Nq = not quantifiable.

aromas (6.8 and 15.4) increased greatly in intensity in sample C as compared with their intensities in sample B.

Group IV (floral, green, solvent-like) exhibits a disparate pattern. One reason for this could be that the group in fact is not one group, but is made up of several different aromas. Many aromas are present in sample A only (5.9, 20.7, 22.8, 25.3, and 25.6). One representative of this group (10.4) has been identified as hexanal and remains at approximately the same aroma intensity throughout the process. Another identified compound that could as well be classified into group V, being an ester, methyl-2-methylpentanoate (18.5), was present only in sample C.

In Group V, the fruity, solvent-like, and ester-like notes in general exhibited high aroma intensities at the beginning of the process (sample A) and had disappeared by sample B. The aroma 29.5, however, was present only in sample B. With the exception of 9.5, the aromas from group V were not present in sample C.

Liquid-Liquid Extraction. The results from the GC-O analysis for the liquid-liquid extracts are summarized in Table 3. Samples A and C exhibited 16 and 15 aromas, respectively, and sample B exhibited 10 aromas. The aromas from group I were detected only in sample A. A number of carboxylic acids were identified and found responsible for these aromas (9.5, 12.2, 15.0, 16.5, and 32.7). The finding that sour aromas, caused by carboxylic acids, disappear in the process after the raw juice is in agreement with the results for sour aromas extracted by gas phase. In an earlier study of diethyl ether extracts of samples from different stages

of raw sugar manufacturing and refining the same pattern was observed (2). An explanation for this could be that acids are neutralized in the carbonation steps prior to the refinery syrup (sample B) and liquid sugar (sample C) stages.

The pattern in which an aroma was perceived in sample A but not in samples B and C was also found for the earthy aromas of group II. Geosmin was identified by means of MS (its base peak is at $m/z = 112$) and exhibited an earthy, potato-like aroma (30.6).

For the caramel-like aromas, group III, the trend from the headspace analyses, namely that these aromas were developed in the later stages (in either, or both, of samples B and C) of the refining process, was even more emphasized for the diethyl ether extracts. Also, some of the caramel aromas were perceived at comparatively high intensities in sample C, the sample which had passed more purifying steps and therefore should have been the cleanest. An exception to the described trend were 17.5 and 31.8, which were perceived only in sample A.

Two general trends were observed for the floral notes (group IV). One trend was that some aromas (27.9, 34.5, and 36.9) were present only in sample A, and the other trend was that some aromas (19.2, 23.4, 26.4, and 29.8) were present only in sample C. One aroma (35.5) was present in samples B and C only. However, groups IV and V are close in aroma quality, so that some of the aromas classed in group IV could just as well have been placed in group V.

General. The retention times for the eluting aroma compounds were different for the gas-phase extraction

and the liquid–liquid extraction. Reasons for this could be the different injection systems and the so-called solvent effect, applicable to the liquid–liquid extracts, that may alter the retention times of the eluting compounds (11). It therefore turned out to be difficult to compare eluting aroma compounds from the two different means of injection. Some aroma compounds from liquid–liquid extraction could, however, be associated with aroma compounds from gas-phase extraction on the basis of retention time, aroma quality, and intensity in the different samples. In group I, a bitter, sour aroma eluting at 9.4 min (referred to as gas-phase-9.4) may correspond to the sour, tennis-shoe-like aroma eluting at 9.5 minutes (referred to as liquid–liquid-9.5). An old plastic, rubber-like aroma, gas-phase-24.9, and an earthy, moldy aroma, gas-phase-31.0, belonging to group II, correspond to a floral, and grass- and solvent-like aroma (liquid–liquid-24.9) and an earthy, potato-like aroma identified as geosmin (liquid–liquid-30.6), respectively. Another possible match in group II is an earthy, moldy, plant-like aroma, gas-phase-26.1, identified as nonanol, and the earthy aroma liquid–liquid-27.3, even if the difference in retention time is quite large. Possible matches in group III are gas-phase-19.2 and liquid–liquid-18.4, together with gas-phase-20.6 and liquid–liquid-19.6, and gas-phase-33.1 and liquid–liquid-33.0, determined on the basis of mass spectrometric similarities. In group IV, gas-phase-19.7 corresponds well with liquid–liquid-19.2, and gas-phase-22.8 corresponds well with liquid–liquid-23.4, as the detected compounds exhibit similarities in MS.

An important question raised by the findings is why the intensity of some compounds was increased, instead of decreased, when passing from the refinery sample B to the final product, sample C. This phenomenon has been encountered before, when the concentration of volatiles in diethyl ether and gas-phase extracts was measured for different samples along the process line of sugar manufacturing and refining (2). In that study, the increase was observed for 2,6-dimethylpyrazine, 2-ethylhexanol, methyl pyrrole ketone, and 3-ethyl-2,5-dimethylpyrazine when extracting with diethyl ether, and for propanone, 2-methylpropanal, butanone, 2,3-pentadione, 3-pentanone, 3-hydroxy-2-butanone, dimethyl disulfide, 2-hydroxy-3-pentanone, methylpyrazine, 2,6-dimethylpyrazine, 2-ethyl-5-methylpyrazine, nonanal, and decanal when extracting with gas. In the present investigation, in which fewer compounds were identified, this pattern can be observed for the aromas of 2-ethyl-5,6-dimethylpyrazine, butanone, 3-hydroxy-2-butanone, dimethyl disulfide, 2-methylpropyl acetate, 2,5-dimethylpyrazine, and methyl-2-methylpentanoate. From these results, it follows that not only did some compounds increase in concentration in the final product (i.e., sample C) but the aromas released from the solution also increased in intensity. These are interesting findings, considering that in the sensory evaluation of the total aromas of sample B and C, all assessors rated sample B at least two units higher than sample C, thereby saying that sample B had a more intense total aroma than did sample C. This would imply that the overall aroma was caused by some other, not detected, compound, and that in the investigated samples, the large number of ketones and other compounds enumerated are perhaps not aroma-active at their concentrations. One reason for the increase in volatile compounds in sample C in comparison with

those in sample B could be that the activated carbon or the ion exchangers release compounds which they had previously removed. The color and ash content values are, however, at normal operating range, and this speaks against this theory even if it cannot be ruled out. Another explanation could be that other molecules in the less pure solution in sample B, for example those giving rise to color, interact with aroma compounds, preventing them from being transferred to the gas phase or the diethyl ether phase on extraction. This phenomenon has been observed previously for a number of volatile compounds (12). According to these findings, a higher rate of release of volatiles could, then, be expected in sample C, which has a lower content of colorants (see Table 1).

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LITERATURE CITED

- (1) Godshall, M. A.; Grimm, C.; Clarke, M. A. Sensory properties of white beet sugar. *Int. Sugar J.* **1995**, *97*, 296–343.
- (2) Pihlsgård, P.; Larsson, M.; Leufvén, A.; Lingnert, H. Volatile compounds in the production of liquid beet sugar. *J. Agric. Food Chem.* **2000**, *48*, 4844–4850.
- (3) Schnermann, P.; Shieberle, P. Evaluation of key odorants in milk chocolate and cocoa mass by aroma extract dilution analyses. *J. Agric. Food Chem.* **1997**, *45*, 867–872.
- (4) Ullrich, F.; Grosch, W. Identification of the most intense odor compounds formed during autoxidation of linoleic acid. *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 277–282.
- (5) Acree, T. A.; Barnard, J.; Cunningham, D. A procedure for the sensory analysis of gas chromatographic effluents. *Food Chem.* **1984**, *14*, 273.
- (6) Miranda-Lopez, R.; Libbey, L. M.; Watson, B. T.; McDaniel, M. R. Odor analysis of pinot noir wines from grapes of different maturities by a gas chromatography-olfactometry technique (Osme). *J. Food Sci.* **1992**, *57*, 985–993.
- (7) Hall, G.; Andersson, J.; Lingnert, H.; Olofsson, B. Flavour changes in whole milk powder during storage III. *J. Food Qual.* **1985**, *7*, 153–190.
- (8) Pihlsgård, P.; Lingnert, H.; Leufvén, A.; Larsson, M. Aroma analysis of liquid sugars. *Int. Sugar J.* **1998**, *100*, 431–436.
- (9) Marsili, R. T.; Miller, N.; Kilmer, G. J.; Simmons, R. E. Identification and quantitation of the primary chemicals responsible for the characteristic malodor of beet sugar by purge and trap GC-MS–OD techniques. *J. Chromatogr. Sci.* **1994**, *32*, 165–171.
- (10) Arctander, S. *Perfume and Flavor Chemicals*. Monclair, NJ, 1969.
- (11) Jennings, W. *Analytical Gas Chromatography*. Academic Press, Inc.: San Diego, CA, 1993.
- (12) Pihlsgård, P.; Leufvén, A.; Lingnert, H. The influence of macromolecules on aroma release in liquid sugar. Submitted for publication, 2000.

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