

Volatile Compounds in the Production of Liquid Beet Sugar

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Samples from different parts of a beet sugar factory and refinery were analyzed with respect to volatile compounds by means of liquid–liquid extraction followed by gas chromatography–mass spectrometry (GC-MS). A limited number of the samples were analyzed by means of gas phase extraction (headspace) followed by GC-MS. Selected compounds were followed through the sugar manufacturing process. The behavior of different compounds varied greatly throughout the process, with some compounds such as geosmin (*trans*-1,10-dimethyl-*trans*-9-decalol), dimethyl disulfide, and propionic and hexanoic acid present at the beginning of the process but disappearing rapidly after further processing. Other compounds, such as indole, dihydrobenzofuran, and 2-phenylethanol, were not detected at the start of the process but were formed later on and removed in the final product. In the final product, three pyrazines remained at fairly low concentrations, together with 3-methylcyclopentadione, ethylhexanol, and methyl pyrrole ketone.

Keywords: Beet sugar; sugar refining; volatile; flavor; GC-MS

INTRODUCTION

There are many different sources of volatile compounds that appear in the process of beet sugar manufacture. Volatile compounds often give rise to odors and flavors, and if allowed to remain in the final product, they limit its application. Some compounds come from the sugar beet itself, either as a consequence of uptake from the surrounding soil or as a result of microbial action or the beet's own metabolism. Others come from the actual process of sugar manufacture and refining (Godshall et al., 1995).

Earthy and musty odors are often associated with odor problems of beet sugar. The molecule *trans*-1,10-dimethyl-*trans*-9-decalol, also known as geosmin, has been held responsible for these earthy, musty notes and has been detected. Other compounds with earthy, musty odors are 2-methoxy-3-*sec*-butylpyrazine (Parliament et al., 1977) and 2-methylisoborneol, the latter detected together with geosmin in agricultural soil (Buttery and Garibaldi, 1976).

Another source of odors and flavors is caramelization products that are formed when sucrose is heated. In this process, both desirable and undesirable odors and flavors are formed. Typical undesirable odors are burnt, bitter, and acrid odors (Monte and Maga, 1981). Many different kinds of volatile compounds are formed by caramelization reactions. Aldehydes, for example, acetaldehyde, propionaldehyde, butyraldehyde, valeraldehyde, and acrolein; ketones, such as acetone, methyl ethyl ketone, and methyl propyl ketone; furans, such as furan, 2-methylfuran, and 2,5-dimethylfuran; and acids, including formic, acetic, and propionic acid, are

examples of this kind of product (Bryce and Greenwood, 1963). Caramelization also gives highly colored compounds.

The Maillard reaction is another major source of volatiles during processing. This reaction between a reducing sugar and an amine in actual fact consists not of one but of thousands of reactions, where each is influenced to a different extent by reactants, pH, water activity, temperature, inhibitors, and so on (Reineccius, 1990). Hence, a great number of different compounds may be formed.

Marsili et al. (1994) analyzed seven samples of beet sugar with various intensities of a characteristic beet sugar odor defect to identify the chemicals responsible for the odor. Geosmin, 2,5-dimethylpyrazine, furfural, butyric acid, and isovaleric acid were judged to be likely contributors to the odor defect. As part of their study, the authors spiked odorless cane sugar with these five candidates in various degrees and combinations to reconstruct the original odor and were successful in imitating it. Godshall et al. (1995) failed to identify geosmin or methylisoborneol through instrumental analysis, and the absence of these compounds was confirmed by gas chromatography–olfactory detection (GC-O) using trained panelists. However, some mushroomy, earthy odors were clearly noted and thought to originate from a number of C₈ alcohols, ketones, and aldehydes (Karahadian et al., 1985).

Using gas chromatography–mass spectrometry (GC-MS) and GC-O, Pihlsgård et al. (1998) analyzed liquid sugars of different degrees of purity, which had passed through several purification steps. Aldehydes, ketones, and pyrazines, together with diacetyl, methoxyphenol, and 2-ethylfuran, were among the identified and quantified compounds. In a later study (Pihlsgård et al., 1999), results of chromatographic data on headspace volatiles from liquid sugars were analyzed for correlation with sensory assessments of the liquid sugars to find out which of the compounds were associated with

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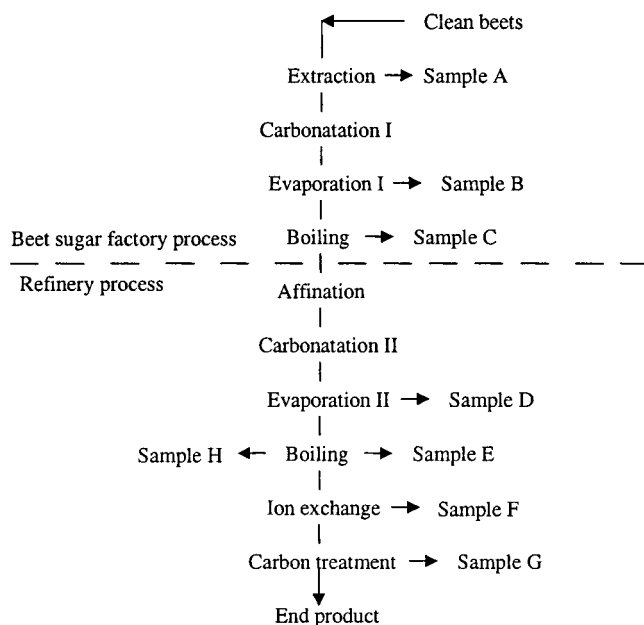


Figure 1. Schematic diagram of raw sugar production and subsequent refining. The samples collected and analyzed are labeled samples A–H.

the different odor notes in the liquid sugars. A number of alkylpyrazines, ketones, and alcohols, together with dimethyl disulfide, 2,5-dimethylfuran, and *p*-methoxyphenol, were among the compounds seen to be critical for different parts of the odor load of the analyzed liquid sugars. This implies that caramel-like or Maillard reaction products as well as compounds from the sugar beet are present in the liquid sugars.

To be able to control the flavor quality of the final liquid sugar product, including finding remedies for sudden quality deviations and finding suitable applications for the product, the behavior of odoriferous compounds through the different process steps must be known. Improvement of the product by adding or altering process steps will also be facilitated if thorough knowledge is acquired of the role of different odor-active compounds throughout the manufacturing process. This investigation aimed to study the production of liquid sugar, from sugar beet to the final product, with respect to volatile compounds. Samples from different sections of the liquid sugar production were extracted with diethyl ether prior to analysis by GC-MS. The presence and level of the tentatively identified volatile compounds were determined in the different samples along the process line, with the solution passing through several important purification steps, and compounds considered to be relevant to the odor and flavor of the final product or to the possibilities of flavor formation in the same were followed through the process.

MATERIALS AND METHODS

Samples. Samples were taken from different parts of Danisco Sugar AB's raw beet sugar factory in Jordberga, Sweden, and the Arlövs sugar refinery, in Arlöv, Sweden (see Figure 1). The cleaned and sliced beets are extracted with hot water (70 °C), and the resulting solution is referred to as "raw juice". The raw juice is treated with lime and carbon dioxide to precipitate CaCO₃ and impurities such as proteins, polysaccharides, and high molecular weight, color-forming compounds, a step referred to as "carbonatation". After filtration, the syrup with a dry substance content of 14–16% is called "thin juice". The thin juice is evaporated, and the resulting syrup with a

dry substance content of 72–74% is referred to as "thick juice". Boiling of the thick juice and subsequent centrifugation to separate the crystals from the residual liquor gives raw sugar that is transported to the sugar refinery for further processing. The raw sugar passes through an affination step for partial removal of the thin layer of nonsugars on the surface of the crystals. The washed crystals are dissolved in water and a carbonation similar to the one described above for the beet sugar factory is performed. The syrup passes first through a decolorizing ion exchanger and then through an evaporator, where the dry substance content is increased from approximately 65 to 78%. Next, the syrup passes a series of boiling steps (performed in consecutive boiling pans) by which white sugar is crystallized in vacuum boiling pans and a mixture of dissolved sugar and residual liquors is recovered for liquid sugar production. This liquor is allowed to pass through a mechanical filter, adsorbent columns, ion exchangers, and columns with activated carbon before it may be used as a liquid sugar.

As shown in Figure 1, in the present study, the raw juice sample (sample A) was taken directly after the extractor at the beet sugar factory and the thick juice sample (sample B) was collected after the evaporator that follows the mechanical filter that filters off the CaCO₃ from the carbonation process. The sample of raw sugar (sample C) was taken at introduction of the material into the refinery. The material for boiling (sample D) was collected after the evaporators, just before the first vacuum pan. Unified streams from the different boiling pans (dissolved sugar and syrups) are collected in a tank. Sample E was taken from that tank. Sample F was taken after the adsorbent column and the ion exchanger, and sample G was taken after the carbon columns. The last boiling pan is where nonsugars are enriched. Residual syrup from this pan is referred to as "molasses". A molasses sample (sample H) was collected to verify the increase of certain impurities.

The whole sampling series was performed on two different occasions, at the beginning of the beet campaign in 1998 (mid-September) and toward the end of the campaign (mid-December). The first sampling series will be referred to as "series 1" and the second as "series 2". Prior to analysis, the samples were stored in glass bottles at –40 °C.

Sugar Analysis. All investigated samples were analyzed for dry substance content, invert sugar content, pH, color, and ash content according to International Commission for the Uniform Methods of Sugar Analysis (ICUMSA) methods. The invert sugar and ash contents referred to are given as the ratio between the amount of the respective parameter and the dry substance content on a weight basis (referred to as "quota" in Table 1).

Liquid–Liquid Extraction Procedure. A volume of 80 mL of sugar solution (40 mL diluted with 40 mL of Millipore water, if the dry substance content of the sample exceeded 30%) was placed in a separatory funnel. Raw juice samples were filtered through glass wool prior to extraction to remove particles that could disturb the extraction. The sugar solution was extracted three times with 10 mL portions of diethyl ether, which were united and dried over sodium sulfate. Two internal standards, octanol and dodecane, were added to the diethyl ether prior to extraction to control the extraction and evaporation processes. The dried extract was placed in a flask with a Vigreux-type column and immersed in a water bath at 35 °C, and the solvent was allowed to evaporate to concentrate the sample to a final volume of ~100 µL. Before the actual analysis was performed, a droplet of extract was applied on a strip of filter paper. After the solvent had evaporated, the paper was smelled so as to ensure that the odor of the extract was the same as the odor of the sample which the extract had come from. The extracts were stored in capped vials at –20 °C prior to analysis. All samples were extracted and analyzed in triplicate.

Gas Phase Extraction Procedure. Samples B, E, and G (Table 4) from series 1 were investigated by using a gas phase extraction technique, and results were compared with those from liquid–liquid extraction. Volatile compounds from the liquid sugar samples were adsorbed on Tenax (60–80 mesh

Table 1. Results of the Analyses According to ICUMSA Methods^a

sample	RDS%	invert sugar (quota)	ash content (quota)	pH	color/ICU
A	16.0/15.8	17.0/16.6	0.350/0.926	4.5/3.9	2290/2270
B	72.1/73.1	0.081/0.172	1.79/1.69	9.6/9.2	2565/2952
C	na	na	na	na	na
D	77.0/78.0	0.04/0.03	0.05/0.04	7.0/7.1	44/42
E	63.0/61.7	0.166/0.268	0.505/0.429	9.0/7.6	1610/1241
F	57.6/58.8	0.141/0.212	0.004/0.008	7.0/7.9	33/231
G	62.3/61.6	0.183/0.310	0.004/0.007	7.5/7.0	262/264

^a Results are given for series 1/series 2. Data for the solid raw sugar (sample C) are not available.

in a glass cartridge) according to the method described by Hall et al. (1985). Each sample (100 g in a 500 mL flask) was allowed to equilibrate for 30 min at 40 °C. Thereafter, 5 L of helium was allowed to pass through the flask and the cartridge with adsorbent material at a rate of 40 mL/min. The solution was stirred during equilibration and headspace sampling.

GC-MS Analysis. The diethyl ether extracts were injected by means of an HP 7683 automatic injector into an HP 6890-5973 GC-MS (Hewlett-Packard, Rockville, MD) equipped with a 60 m × 0.32 mm capillary column with a 1.0 μm thick film of DB-1 (J&W Scientific Inc., Folsom, CA). The injected volume was 1 μL. The initial temperature of the chromatographic oven was 35 °C. The temperature was raised to 220 °C at a ramp rate of 4 °C/min and then held at 220 °C for 10 min. Identification and integration of the GC peaks were done with the computer software Chemstation (Hewlett-Packard), and quantification was done on the basis of the internal standard dodecane, as this compound gives a sharper and more resolved GC peak than does octanol. The concentration of each compound was related to the dry substance content in the particular sample.

The Tenax cartridges with trapped headspace volatiles were thermally desorbed prior to injection into the GC equipment by means of an ATD 400 automatic injector (Perkin-Elmer, Norwalk, CT). The desorption temperature was 250 °C, and the desorption time was 5 min. The gas chromatograph used was a Varian 3200 (Varian Inc., Palo Alto, CA) equipped with a column equivalent to that used for extracts; the temperature programming was identical to that described for extracts. The effluent from the capillary column was split 1:3, with one-fourth routed to an Inco 50 mass spectrometer (Finnigan, San Jose, CA) and three-fourths directed to a flame ionization detector. Integration on the basis of flame ionization detection (FID) was done with a Hewlett-Packard 3550 laboratory data system, and the levels of volatile compounds were determined by using the external standard nonane.

RESULTS AND DISCUSSION

Sugar Analyses. The results of the sugar analyses are shown in Table 1. There was a generally low level of invert sugar content except in the raw juice, where levels were very high. The ash quota [i.e., the ash content in relation to the refractometric dry substance (RDS)] was highest in the thick juice and lowest toward the end of the production line. The pH usually varies considerably, but in general, the raw juice (sample A) has a low pH (of ~4), and the thick juice (sample B), which follows a carbonatation step where the addition of lime increases the pH, has a high pH (of >9). The solution for liquid sugar production (sample E) exhibited a high pH (9.0) in series 1 and a moderate pH (7.6) in series 2. The color load decreased as the end of the process line was approached. A slight increase in color was noticed in the final product (sample G) as compared with the solution after the ion exchangers (sample F).

Liquid-Liquid Extraction Gas Chromatography. Because the chromatograms, especially of extracts

from the less pure samples, were very complex, the number of compounds to be studied had to be reduced. In the end, peaks of 20 different compounds, chosen largely on the basis of previous knowledge about the liquid beet sugar system, were studied in greater detail. Carboxylic acids have been claimed to be responsible for odor in beet sugar (Godshall et al., 1995; Marsili et al., 1994), so propionic and hexanoic acid were followed through the process, as well as 2,6-dimethylpyrazine, 2-ethyl-5-methylpyrazine, and 3-ethyl-2,5-dimethylpyrazine, all three of which have been detected and found to be correlated with odors in liquid sugar (Pihlsgård et al., 1999). Alkoxy-pyrazines have been reported to cause an earthy/musty aroma (Maga, 1987), so 2-methoxy-3-(1-methylpropyl)pyrazine was chosen for the study, as well as 3-methyl-cyclopentadione, also known as "cyclotene", which has been identified as a product of sugar degradation (Nishimura et al., 1980). Two C₈ alcohols have previously been reported to be correlated to odors and flavors in liquid sugar (Pihlsgård et al., 1999), so we also followed another compound, 2-ethyl-hexanol, through the production process. We also studied the behavior of methyl pyrrole ketone, as pyrrole derivatives are often formed in the Maillard reaction and as it has been reported that this particular compound can form as a result of glucose cooked with serine (Baltes et al., 1989). Our list also included 2-phenyl-ethanol, which is known to have a very potent odor and which is often used in perfumes (Arctander, 1969). Dimethyl disulfide has been found in liquid sugar (Pihlsgård et al., 1998, 1999) and is thought to be formed via the reaction between a reducing sugar and a sulfur-containing amino acid (Lindsay, 1985). Other compounds studied included dihydrobenzofuran, indole, and 1*H*-indole-3-carboxaldehyde. These were chosen out of curiosity because they were present in some samples but have not, to our knowledge, been discussed in the literature in relation to liquid sugar. The formation of 1-methyl-1*H*-pyrrolecarboxaldehyde as a product of the Maillard reaction has been observed by Nyhammar et al. (1983); hence, this compound was followed through the process. Phenolic compounds, which include 2-methoxy-4-vinylphenol, 4-hydroxybenzaldehyde, vanillin, and 4-hydroxybenzenethanol, have been separated and investigated in beet sugar molasses (Godshall, 1986) and were therefore included in the list of compounds studied. The examined compounds, together with their concentration in the extraction medium, based on the sugar content, are shown in Tables 2 (series 1) and 3 (series 2).

The compounds 2-methoxy-3-(1-methylpropyl)pyrazine, dimethyl disulfide, and geosmin were present only in sample A, the raw sugar sample. Propionic acid was only present in sample A and the molasses sample (sample H). In series 1, the concentration of propionic acid was much higher than in series 2, indicating microorganism activity (Clarke et al., 1991). The concentrations of hexanoic acid and cyclotene were much higher in sample A than in the other samples, which, for hexanoic acid, was probably due to the activity of microorganisms. In the case of cyclotene, the high concentration in the raw juice is more difficult to explain because this molecule has been reported to be a sugar degradation product (Nishimura et al., 1980). Several actinomycetes, especially the streptomyces, are known to produce geosmin (Gerber and Lechevalier, 1965). The actinomycetes are widely distributed in nature and are

Table 2. Concentrations (Nanograms per Gram of Sugar) of the Studied Volatile Compounds (Liquid-Liquid Extraction), Series 1^a

compound	A	B	C	D	E	F	G	H
propionic acid	10600	0	0	0	0	0	0	867
2,6-dimethylpyrazine	335	749	28.5	143	435	381	528	413
hexanoic acid	237	0	3.85	0	0	6.35	0	0
2-ethyl-5-methylpyrazine	35.2	330	0.880	92.9	540	450	13.6	265
3-methyl-cyclopentadione	2010	21.8	24.6	8.71	7.69	38.2	7.63	319
2-ethylhexanol	18.8	87.5	77.9	95.7	102	0	43.6	91.3
methyl pyrrole ketone	33.7	11.9	0	0	27.0	0	23.4	0
3-ethyl-2,5-dimethylpyrazine	0	67.4	0.749	6.17	20.3	40.8	307	208
2-phenylethanol	0	11.2	0	0	1.98	0	0	0
2-methoxy-3-(1-methylpropyl)pyrazine	41.6	0	0	0	0	0	0	0
dimethyl disulfide	2.94	0	0	0	0	0	0	0
dihydrobenzofuran	0	154	0	54.4	46.3	0	0	0
indole	0	10.8	1.42	21.3	20.7	7.44	0	1570
1-methyl-1 <i>H</i> -pyrrolecarboxaldehyde	20.8	81.2	0	17.5	41.7	4.69	0	0
2-methoxy-4-vinylphenol	16.5	28.2	4.07	49.8	23.8	6.16	0	1110
4-hydroxybenzaldehyde	3.57	24.9	7.63	17.6	60.3	6.78	0	2010
vanillin	148	49.6	17.9	13.4	28.1	119	0	1570
4-hydroxybenzene-ethanol	57.5	164	0	8.33	42.4	0	0	0
geosmin	43.4	0	0	0	0	0	0	0
1 <i>H</i> -indole-3-carboxaldehyde	0	61.9	0	48.3	88.6	22.5	0	2870

^a A, raw juice; B, thick juice; C, raw sugar; D, solution for boiling; E, solution for liquid sugar production; F, solution after ion exchange; G, final product; H, molasses.

Table 3. Concentrations (Nanograms per Gram of Sugar) of the Studied Volatile Compounds (Liquid-Liquid Extraction), Series 2^a

compound	A	B	C	D	E	F	G
propionic acid	884	0	0	0	0	0	0
2,6-dimethylpyrazine	719	730	9.85	30.7	136	397	90.9
hexanoic acid	188	0	6.10	0	0	0	0
3-ethyl-5-methylpyrazine	92.7	298	9.05	14.3	105	196	0
3-methylcyclopentadione	2410	52.3	18.7	5.37	10.9	18.5	2.67
2-ethylhexanol	0	148	40.0	52.1	33.1	0	0
methyl pyrrole ketone	24.0	8.65	0	0	17.6	0	0
3-ethyl-2,5-dimethylpyrazine	144	131	4.22	0	15.5	24.6	103
2-phenylethanol	0	8.69	0	0	0	0	0
2-methoxy-3-(1-methylpropyl)pyrazine	37.9	0	0	0	0	0	0
dimethyl disulfide	6.24	0	0	0	0	0	0
dihydrobenzofuran	0	125	0	23.2	48.5	0	0
indole	0	21.3	5.85	12.1	17.5	8.12	0
1-methyl-1 <i>H</i> -pyrrolecarboxaldehyde	0	97.7	3.57	8.34	31.4	0	0
2-methoxy-4-vinylphenol	45.5	486	25.6	31.2	26.3	0	0
4-hydroxybenzaldehyde	1.04	56.5	18.4	6.84	100	0	0
vanillin	467	129	50.1	17.9	368	7.96	0
4-hydroxybenzene-ethanol	29.7	125	0	2.74	50.8	0	0
geosmin	45.8	0	0	0	0	0	0
1 <i>H</i> -indole-3-carboxaldehyde	0	50.5	27.7	61.5	120	0	0

^a A, raw juice; B, thick juice; C, raw sugar; D, solution for boiling; E, solution for liquid sugar production; F, solution after ion exchange; G, final product.

quite common in soil, with their metabolites giving soil its earthy smell (Maga, 1987). Murray and Whitfield (1975) surveyed the presence of 3-alkyl-2-methoxypyrazines in raw vegetable tissue, and beetroot was one of the vegetables found to contain this group of compounds. Certain alkoxy-pyrazines exhibit earthy/musty flavors (Parliament and Epstein, 1973).

In the thick juice sample (sample B), the concentration of the alkylpyrazines was greater than in the raw juice sample. This trend was more obvious in series 1 than in series 2. The explanation for the increase in the thick juice sample is the formation of alkylpyrazines when sugar is thermally degraded, as in carbonatation reactors (Shibamoto, 1989). Thus, the concentration of 2-ethylhexanol was increased in the thick juice sample, and 2,6-dimethylpyrazine, 2-phenylethanol, 1,2-dihydrobenzofuran (coumaran), and 1-methyl-1*H*-pyrrolecarboxaldehyde were at their highest concentrations in the thick juice sample. For 2-methoxy-4-vinylphenol, the concentration in series 2 was >10 times higher than in series 1. Part of this can be explained by the fact that

the concentration of 2-methoxy-4-vinylphenol was 3 times higher in the raw juice sample of series 2 than in the corresponding sample of series 1. The conditions of the carbonatation and evaporation steps between the raw juice sample and the thick juice sample are not always constant, and this could explain the larger difference in concentration for the thick juice samples. Likewise, there was a large increase in the concentration of 4-hydroxybenzaldehyde, 4-hydroxybenzene-ethanol, and 1*H*-indole-3-carboxaldehyde; however, vanillin was reduced to approximately one-third of its concentration in the raw juice sample.

The raw sugar sample (sample C) in both series had very low levels of volatile compounds compared with the previous samples. As sugar is stored between production and refining, sometimes for up to 8 months, the low levels of volatile compounds may have been due to aeration, which has been shown to reduce volatile compounds in sugar (Clarke et al., 1991). Hexanoic acid, which was not detected in the thick juice sample, was detected in the raw sugar in both series 1 and 2. The

concentration of the three alkylpyrazines was much lower in the raw sugar than in the thick juice sample. The decrease in concentration of cyclotene, 2-ethylhexanol, vanillin, and 1*H*-indole-3-carboxaldehyde (series 2 only) between the thick juice sample and the raw sugar sample was smaller than for most compounds that were heavily reduced in the raw sugar.

The sugar analyses showed that the solution for boiling (sample D) had very low color values, indicating a low color load, which suggests a low concentration of sugar degradation products. Nevertheless, the alkylpyrazines all had an increased concentration compared with the alkylpyrazines in raw sugar. The increase in concentration of the analyzed compounds from sample C to sample D was less pronounced for series 2 than for series 1, in which the concentrations of dihydrobenzofuran, indole, 1-methyl-1*H*-pyrrolicarboxaldehyde, 2-methoxy-4-vinylphenol, 4-hydroxybenzene-ethanol, and 1*H*-indole-3-carboxaldehyde increased significantly. Furthermore, for series 1, the concentrations of hexanoic acid and cyclotene were decreased to zero and reduced to one-third, respectively.

The solution for liquid sugar production (sample E) consists of unified streams from the boiling pans and, compared with sample D, it is expected to be much more loaded with compounds giving odor and color. Most of the studied compounds increased their concentration between sampling point D and sampling point E. The compounds for which the concentration increased the most were the alkylpyrazines, methyl pyrrole ketone, 1-methyl-1*H*-pyrrolicarboxaldehyde, 4-hydroxybenzaldehyde, vanillin (significantly more for series 2 than for series 1), 4-hydroxybenzene-ethanol, and 1*H*-indole-3-carboxaldehyde. However, the concentration of 2-methoxy-4-vinylphenol was lower in sample E than in sample D.

The syrup that had passed through the ion exchanger (sample F) lost much of the brown color of sample E, which should imply that the odor load and, hence, the concentration of volatile compounds are also lower. The compounds with the greatest decrease in concentration between sampling points E and F were 2-ethylhexanol, methyl pyrrole ketone, dihydrobenzofuran, 1-methyl-1*H*-pyrrolicarboxaldehyde, 2-methoxy-4-vinylphenol, 4-hydroxybenzene-ethanol, and 1*H*-indole-3-carboxaldehyde. The compounds 2-phenylethanol, indole, and 4-hydroxybenzaldehyde decreased moderately. At the same time, contrary to expectations, the concentration of some compounds increased between the two sampling points. This could be explained by a reduced capacity of the adsorbents or ion exchangers and possibly a release of compounds already bound. The compound 2,6-dimethylpyrazine remained unchanged in series 1 but increased 3-fold in series 2; cyclotene increased in concentration for both series, as did 3-ethyl-2,5-dimethylpyrazine; and the concentration of vanillin increased for series 1 but decreased for series 2. The large discrepancy between the contents of volatile compounds in sample F in the two series may also be observed by studying the large differences in color load for this sample (Table 1).

When the syrup passed from sampling point F through columns with activated carbon to point G, some volatile compounds such as indole, 1-methyl-1*H*-pyrrolicarboxaldehyde, 2-methoxy-4-vinylphenol, 4-hydroxybenzaldehyde, vanillin, and 1*H*-indole-3-carboxaldehyde decreased to levels at which the detector could no longer

distinguish them. Others, such as 2-ethyl-5-methylpyrazine, 2-ethylhexanol, and methylpyrrolketone, could not be detected in series 2, despite the fact that series 1 showed them to be present at fairly high levels.

Some compounds increased from sampling point F to point G. For instance, 2,5-dimethylpyrazine increased moderately in series 1, although it decreased in series 2; 2-ethylhexanol and methyl pyrroleketone increased in series 1 but remained unchanged (*viz.*, at zero) for series 2. The compound 2-ethyl-5-methylpyrazine was reduced in both series; in series 2, its level was down to zero, whereas a small amount was still detected in series 1. A compound that increased significantly between the two sampling points was 3-ethyl-2,5-dimethylpyrazine.

To summarize, the most abundant compounds in the final product were 2,6-dimethylpyrazine, 2-ethyl-5-methylpyrazine, cyclotene, 2-ethylhexanol, and methyl pyrrole ketone (the latter two in series 1 only) and 3-ethyl-2,5-dimethylpyrazine.

It is possible that odorous compounds are formed from ion-exchange resins if damaged by osmotic shock or operation at too high a temperature. Resins may also develop odors when remaining unused, as in down times, and the compounds responsible for these odors may give off a very strong odor in the first bed volume (M. A. Godshall, personal communication, April 1996).

The final product, collected at sampling point G, after it had passed through columns filled with activated carbon, still contained some of the investigated volatile compounds, mainly 2,6-dimethylpyrazine and 3-ethyl-2,5-dimethylpyrazine. The concentrations of these compounds were significantly higher in series 1 than in series 2. In fact, the concentration of 2,6-dimethylpyrazine had, for series 1, remained unaffected despite passage of the material through adsorbents, ion exchangers, and activated carbon. The other compounds investigated along the process of beet sugar processing and liquid sugar manufacture were not detectable in this solution. It should be emphasized, however, that even if a compound such as geosmin has been removed to a level at which it cannot be detected, it may still cause an odor in the final product because its odor threshold may be very low.

White crystal sugar is very pure and does not have an odor. All impurities have been removed and are enriched in the molasses. Hence, the refinery molasses (sample H) should contain high levels of the impurities present in the raw sugar fed into the refinery. Of the investigated volatile compounds, the refinery molasses were dominated by propionic acid, 2,6-dimethylpyrazine, cyclotene, 2-ethylhexanol, indole, 2-methoxy-4-vinylphenol, 4-hydroxybenzaldehyde, vanillin, and 1*H*-indole-3-carboxaldehyde. On the basis of their sugar content, the concentrations of some of these compounds were up to 30 times higher in the molasses than in the raw sugar. It is interesting to note, however, that compounds with earthy, musty odors, such as geosmin and 2-methoxy-3-(1-methylpropyl)pyrazine, were not detected in the molasses sample. These compounds must have been efficiently removed by carbonatation, which takes place prior to the boiling steps during which molasses are recovered.

To form an idea of the uncertainties in the measurements, Table 5 gives the relative standard deviation (RSD) for the peaks of some of the compounds studied. No integrations exhibited a higher RSD than the highest RSD quoted in Table 5.

Table 4. Concentrations (Nanograms per Liter of Headspace) of the Studied Compounds (Gas Phase Extraction), Series 1^a

compound	B	E	G
acetaldehyde	4.24	12.0	9.25
propanone	30.9	62.9	200
2-methylpropanal	8.25	73.2	83.8
diacetyl	14.5	43.8	22.0
butanone	6.84	52.0	239
2,3-pentadione	11.3	10.9	19.7
3-pentanone	0	1.94	22.5
3-hydroxy-2-butanone	6.63	25.1	54.3
dimethyl disulfide	31.9	108	178
2-hydroxy-3-pentanone	0	2.48	12.2
methylpyrazine	11.1	16.5	26.2
2,6-dimethylpyrazine	700	227	322
benzaldehyde	2.49	4.47	3.60
6-methyl-5-hepten-2-one	8.91	8.83	0.807
2-ethyl-5-methylpyrazine	154	154	94.2
nonanal	13.2	12.9	19.1
decanal	14.6	17.4	24.0

^a For the various sampling points B, E, and G, see Figure 1.

Table 5. Relative Standard Deviations (RSD) for Measurements of the Concentration of Selected Compounds^a

compound	RSD (%)
2,6-dimethylpyrazine	6.5
2-ethyl-5-methylpyrazine	6.2
3-ethyl-2,5-dimethylpyrazine	39
vanillin	48
indole	33
4-hydroxybenzaldehyde	36

^a $n = 3$.

Gas Phase Extraction GC. The results of analysis of the volatile profile in samples B, E, and G from series 1 by means of gas phase extraction were quite different from those obtained through liquid–liquid extraction. The first obvious feature of the results of the gas extractions was that the compounds encountered in the chromatograms were not the same as those found in the liquid–liquid extracts. Therefore, the compounds studied were not the same as for liquid–liquid extraction even if some compounds were encountered after both extraction procedures. Because the number of sampling points was fewer and only one series of samples was analyzed, a comparison between the extraction procedures is not always easy.

The compounds analyzed in the liquid–liquid extracts, which were also present in the gas extracts, were dimethyl disulfide (only present in liquid–liquid extracts from raw juice), 2,6-dimethylpyrazine, and 2-ethyl-5-methylpyrazine. It is interesting to note that the trends between samples is that the concentration increased, rather than decreased, from the thick juice state to the final product. Throughout the refinery process, the alkylpyrazines decreased through the process but still ended up in the final product at relatively high levels. By contrast, the concentration of dimethyl disulfide increased through the process.

There are some important features that need to be explained. One is the presence of small ketones, such as propanone, butanone, 3-pentanone, 3-hydroxy-2-butanone, and 2-hydroxy-3-pentanone. These compounds were not detected in the liquid–liquid extract, even when extracts from liquid sugar samples spiked with selected ketones at concentrations typical for the actual samples were analyzed. This indicates that either

the ketones studied stay in the water phase or they were not detected by the mass spectrometer, a theory that seems less probable because ketones from gas phase extraction were clearly detected by mass spectrometry (Pihlsgård et al., 1998, 1999).

An interesting observation is that gas phase extraction showed all five ketones studied to increase in concentration in the material as it passed from the thick juice state to the final product and that their concentrations in the final product were in the same range as those of the alkylpyrazines. Diacetyl and benzaldehyde were at their highest concentration in the solution for liquid sugar production, and 2,3-pentadione increased through the liquid sugar process. Nonanal and decanal both increased in concentration through the process.

The different pyrazines, methyl-, 2,6-dimethyl-, and 2-ethyl-5-methylpyrazine, were detected at high concentrations in the three samples B, E, and G. The headspace concentration of methylpyrazine was higher in the solution for liquid sugar production than in the thick juice and even higher in the final product. The concentration of 2,6-dimethylpyrazine was very high in the thick juice and still rather high in the final product, even though it had been reduced to half of the concentration in the thick juice; 2-ethyl-5-methylpyrazine was slightly reduced in the final product compared with the thick juice. The ketones, such as propanone, butanone, 3-pentanone, 3-hydroxy-2-butanone, exhibited a higher concentration in the final product than in the thick juice, which was a little surprising. The compounds 2,3-pentadione and diacetyl (butanedione) likewise exhibited higher concentrations in the solution for liquid sugar production and the final product than in the thick juice. Both the short aldehydes, such as acetaldehyde, and the longer aldehydes, such as nonanal and decanal, were present at higher concentrations in the solution for liquid sugar and in the final product than in the thick juice.

A comparison between the results of the liquid–liquid and the gas phase extractions shows that not only are different compounds detected with gas phase extraction but their behaviors through the process also differ. Although the concentration of most compounds investigated by means of liquid–liquid extraction methods seemed to decrease through the process, the compounds detected and investigated by means of gas phase extraction appeared to increase in concentration. It should be remembered that what is detected by the GC detector is dependent on what is injected into the detector in the first place. Furthermore, what is injected is dependent on what is extracted from the original sample. Obviously, in the case of liquid–liquid extraction and gas phase extraction, the means of extraction are totally different. In liquid–liquid extraction, the solution is extracted with an organic solvent, in this case diethyl ether. The compounds extracted are, apart from the actual content in the sample to be analyzed, dependent on the choice of solvent and a number of other factors such as the concentration of the original solution at the time of extraction, volume of solvent, duration of the extraction procedure, and temperature. In the case of gas phase extraction, critical factors are the adsorbent material and the amount of this material, the volume of gas allowed to flow through the sampling vessel, and the flow rate of the gas as well as the temperature. Also, the amounts of sample injected into the GC detector are different in the two methods.

Conclusions. The process of sugar manufacture is very complex and includes a recycling of material, which explains why the concentration of most of the volatile compounds studied varied considerably through the process. The fact that this study was performed on samples produced in an industry process may explain deviations between the two sampling series. Of the studied compounds, 2,6-dimethylpyrazine, 2-ethyl-5-methylpyrazine, 3-methylcyclopentadione, 2-ethylhexanol, methyl pyrrole ketone, and 3-ethyl-2,5-dimethylpyrazine were detected in the final product. Some compounds such as 2-phenylethanol, dihydrobenzofuran, indole, and 1*H*-indole-3-carboxaldehyde were not present at the start but developed further down the process. Others, such as geosmin and propionic and hexanoic acid, were present in the beginning but disappeared further along the line. Still other compounds, such as 2-phenylethanol, dihydrobenzofuran, indole, and 1*H*-indole-3-carboxaldehyde, were formed in the process but were removed or at least heavily reduced during later purification steps. Hence, passage through adsorbents, ion exchange, and filtration through activated carbon are efficient means of reducing the volatile compounds discussed.

There are large differences between the results relating to compounds detected through liquid–liquid extraction and results relating to compounds detected by gas phase extraction prior to GC analysis. A significantly greater number of aldehydes and ketones was present in the chromatogram from gas phase extraction than in the liquid–liquid extraction chromatogram. At the same time, the gas phase extraction method did not seem to be as efficient as the liquid–liquid extraction method in picking up the aromatics with heteroatoms, such as pyrroles and furans. Both approaches must therefore be considered if a complete picture is to be obtained.

As was to be expected, the refinery molasses were enriched in nonsugars. Among the compounds followed through the process, indole, 1-methyl-1*H*-pyrrolecarboxaldehyde, 2-methoxy-4-vinylphenol, vanillin, and 1*H*-indole-3-carboxaldehyde, together with some pyrazines and propionic acid, were enriched in the refinery molasses.

SAFETY

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. This was performed in this study.

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