Aroma Changes during Black Currant (Ribes nigrum L.) Nectar Processing

Carsten K. Iversen,*,† Henrik B. Jakobsen,†‡ and Carl-Erik Olsen§

Department of Food Science and Technology, Danish Institute of Agricultural Sciences, Kirstinebjergvej 12, 5792 Aarslev, Denmark, and Chemistry Department, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, 1871 Frederiksberg, Denmark

Aroma changes during processing of black currant nectar were examined both by the dynamic headspace trapping technique and by sensory evaluation. Pasteurized and enzyme-treated nectars were compared with control nectar. Significant differences were found for 13 aroma compounds, of which 10 were subsequently identified. Nine of the identified compounds were esters, and eight of these decreased in quantity during enzyme treatment. Additionally, a total of 14 terpenes or terpenoids were identified, and only one of these changed significantly in concentration during enzyme treatment. Pasteurization caused only minor changes in the concentration levels of the volatile compounds. GC-sniffing was used to estimate the potency of individual aroma compounds; the fruit esters as well as the terpenoids were apparently significant contributors to the aroma of black currant nectar.

Keywords: Aroma; juice; enzymation; pasteurization; black currant

INTRODUCTION

Extensive studies have been made of volatile compounds of black currant berries (Karlsson-Ekström and Sydow, 1973; Sydow and Karlsson, 1971a) and buds (Latrasse et al., 1982; Rigaud et al., 1986; Quere and Latrasse, 1990; Nishimura and Mihara, 1988). Black currant (Ribes nigrum L.) has glandular trichomes containing terpenoids on the surface of leaves, buds, and berries (Enevoldsen, 1991). Black currant has the same terpenoids as expected in berries and buds as well as in the leaves. In addition to numerous terpenoids, black currant berries contain aliphatic esters, carbonyl compounds, and alcohols. The esters are considered to be responsible for the fruity notes, which are absent in the buds and leaves (Latrasse, 1991). The esters are the most general aroma compounds in fruits with the exception of orange, for which terpenoids are absent in the buds and leaves. In addition to numerous terpenoids, black currant berries contain aliphatic esters, carbonyl compounds, and alcohols. The esters are considered to be responsible for the fruity notes, which are absent in the buds and leaves (Latrasse, 1991). They are the most general aroma compounds in fruits with the exception of orange, for which terpenoids are the most common aroma compounds (Schwimmer, 1981).

The impact of an aroma compound can be estimated by the GC-sniffing method. Latrasse et al. (1982) used this method to determine the difference between volatile compounds of primary and secondary importance in the typical black currant aroma. Primary notes were diacetyl, methyl and ethyl butanoate, eucalyptol, and 4-methoxy-2-methyl-2-mercaptobutane. The methoxymercaptobutane contributed with a strong note of cat urine, and Latrasse et al. concluded that this is probably the most important black currant aroma enhancer, although it is present in only a very low concentration in bud extracts. Methoxy-2-methyl-2-mercaptobutane has not yet been identified in black currant juice or nectar.

Aroma changes during heat processing of black currant mash and nectar were examined at the Swedish Institute for Food Preservation Research (SIK) in the early 1970s (Sydow et al., 1971a,b). The main effects of heating were an increase in the concentration of aldehydes and a general decrease in the concentration of terpenoids. The main effect of pressing was a decrease in the content of terpenoids, due to the low solubility of terpenoids in water.

There is no available literature concerning aroma changes during enzyme treatment of black currant nectar, but a study of black currant nectar and black currant wine has been published recently (Leino and Kallio, 1993). The aroma of black currant wine is a result of the action of endogenous and ectolytic enzymes during mash treatment and the action of yeast enzymes during fermentation. A sharp decline in terpenoids was caused by the fermentation process. Both the nectar and the wine aromas were dominated by fruit esters. Some esters declined in quantity during fermentation and others increased.

Enzymes in wine-making have been reviewed by Canal-Llauberes (1993). The interaction of endogenous and exogenous enzymes is complicated and may lead to a number of reactions. Terpenoids can be liberated from glucoside precursors in wine must and in fruit nectar (Gueguen et al., 1996), but in fruit nectar α-glucosidase is inhibited by the high content of fructose and glucose (Canal-Llauberes, 1993). Formation of detrimental six-carbon aldehydes and alcohols can take place, which leads to formation of “grassy” aroma notes (Schreier et al., 1977); enzyme activity from stems and leaves increases these reactions significantly. Ester formation is used as a criterion for the selection of wine yeast strains as only production of “good” esters is desirable (Canal-Llauberes, 1993).
The purpose of this work was to study aroma changes at certain steps during the processing of black currant nectar. This paper reports aroma changes during enzyme treatment and pasteurization of black currant nectar under conditions similar to those applied in the fruit juice industry.

MATERIALS AND METHODS

Nectar Samples. The black currant nectar was prepared from frozen black currant berries of the variety Ben Lomond, harvested mechanically in 1995. The nectar was produced in a pilot plant with a capacity of 200 kg of nectar/h. Berries were thawed overnight. Untreated (control) samples were thawed at 0 °C in a Fryma ML-250 and centrifuged at 20 °C in a screw-strain separator model Siebtechnik at 600 × g. Two milliliters of the enzyme preparation was dissolved in 100 mL water and then added to 35 kg of mash, after which the mash was agitated for 2 min. The mash was treated for 2 h at 50 °C. Pasteurized samples were blended and centrifuged, water and sugar were added, and the nectar was frozen. Enzyme treatment was performed by addition of 0.0057% v/v Pectinex Ultra SP (Novo Nordisk Ferment, Dittingen, Switzerland) with an enzyme activity 8800 PG/mL at pH 3.5. Two milliliters of the enzyme preparation was dissolved in 100 mL water and then added to 35 kg of mash, after which the mash was agitated for 2 min. The mash was treated for 2 h at 50 °C. Pasteurized samples were blended and centrifuged, water and sugar were added, and finally the samples were pasteurized and frozen. The nectar was pasteurized at 88 °C for 27 s in an APV Pasilac plate heat exchanger model 1090 at a flow rate of 180 L/h. Both GC analysis and sensory evaluation were performed on the same day. The nectar was thawed overnight at 5 °C. Nectar production, GC analysis, and sensory evaluation were repeated three times.

Headspace Trapping of Volatiles. After defrosting, 500 g of the black currant nectar was transferred to a 850 mL reaction vessel equipped with a four-flange lid. The temperature in the reaction vessel was kept constant at 18 °C by immersing it in a temperature-controlled water bath. The vessel was made airtight with Teflon stoppers mounted with Teflon tubing (1.58 mm i.d.) and purged for 30 min with helium (285 mL/min). Volatiles were trapped for the following 1 h in glass tubes (4 mm i.d., 180 mm length) filled with 100 mg Porapak Q 50–80 mesh (Waters Inc., Milford, MA) between two silica-gelized glass wool plugs. The helium flow was kept at 285 mL/min throughout the collection period. Volatiles were subsequently eluted from the Porapak columns with 2 mL of glass-distilled methylene chloride (HPLC grade). For quantification, 4-Methyl-1-pentan-3-ol (Aldrich, Steinheim, Germany) was added prior to careful evaporation of excess solvent under nitrogen flow to a final volume of 50 μL.

Purification of Equipment and Purge Gas. The reaction vessel and the lid were deaned with hot running tap water for 3 min following by rinsing in distilled water and drying at 100 °C. After cooling, the inner surfaces of the vessel as well as tubings, stoppers, and Teflon connectors were rinsed with glass-distilled methylene chloride (HPLC grade). Prior to transfer of the nectar to the vessel, the entire purge and trap system was examined for contaminants: The system was purged with helium for 15 min followed by a collection of volatiles in the empty vessel for 1 h using the method described above. After the purge-gas sample had been tested for contaminants, the nectar was transferred to the vessel. After use, the Porapak columns were rinsed with 20 mL of methylene chloride. The last 2 mL of the rinse eluate was concentrated and tested for impurities. Helium for entraining volatiles from the reaction vessel was prefiltered through activated charcoal to remove contaminants.

Gas Chromatography (GC) and Mass Spectrometry (MS). GC was performed on a Shimadzu 14A equipped with a HP-Innovax column (0.25 mm i.d., 12 m, 0.25 μm df). Helium was used as carrier gas, at a flow rate of 1.1 mL/min, head pressure of 130 kPa, and splitless purge time of 45 s (30 mL/min split flow). Injector temperature was 220 °C and FID-detector temperature 220 °C. The oven was programmed as follows: 1.5 min at 32 °C, followed by 3 °C/min to 40 °C, isothermal for 10 min, and 3 °C/min to 220 °C at constant temperature for 10 min. For calculation of Kovats retention indices, linear programming of the oven was applied: 30 °C for 1.5 min and 2.5 °C/min to 220 °C. One microliter of each sample was injected into the GC column in splitless mode (30 mL/min split flow). Compounds were identified by coupled GC/MS on a J EOL JMS A X 550W (JEOL Ltd. Akishima, Tokyo, Japan) mass spectrometer and by an ion trap system, Varian Saturn 2000 mass spectrometer, in combination with a Varian Star 3400CX gas chromatograph. Compounds suggested by the NIST database (NIST 92) were verified by comparison with the retention times of authentic reference compounds. Individual compounds were evaluated by GC-sniffing on a SGE OSS-2 splitter system with humid air purging and helium makeup gas (20 mL/min).

Sensory Evaluation. The sensory panel was trained according to guidelines given in ISO 8586-1 (1993) and ISO 5496 (1992). The descriptive terms were developed by the judges as described in the QDA method (Stone, 1992). Black currant nectar samples were served in brandy glasses, covered with lids, at 20 °C. Only three nectar samples were evaluated at each session because the astringent effect of black currant nectar makes taste evaluation at once quite difficult. Judges were asked to describe both taste and smell, and terms were discussed after the sessions. After three sessions, four terms were chosen for the description of nectar, which was either pasteurized, enzyme treated, or untreated. The chosen terms for both taste and smell were “typical blackcurrant”, “flowerlike”, “stored/fermented”, and “elderberry”. The terms and scale (0–5 point) were trained for five sessions. There were nine judges in the panel.

GC-Sniffing. A Shimadzu 14A gas chromatograph equipped with a 60 m × 0.25 mm (i.d.) HP-Innovax column (HP Inc. 1901N-136) was used for GC-sniffing, which was done with a SGE OSS-2 splitter system with humid air purging and helium makeup gas. The GC conditions were the same as described above. Two judges from the trained sensory panel were chosen. The judges described the odor and classified the intensity as either “weak” or “strong”. Each judge spent 20 min at a time sniffing. The sniffing was repeated twice for each of the judges.

Statistical Analysis. This was done in SAS version 6.04. The ANOVA procedure was used for two-way variance analysis of the sensory data. The ANOVA procedure was used for two-way variance analysis of the GC data; if the P value was below 0.05, the nectar samples were significantly different, and in these cases nectar samples were compared by Duncan's post hoc test. Processing and measurements were repeated three times.

RESULTS AND DISCUSSION

The quantitatively dominating compounds in black currant nectar were the esters, which made up 93% of the volatile compounds shown in Table 1. This is similar to the findings of the Finnish study of black currant nectar (Läno and Kallio, 1993) in which the dynamic headspace technique was also used; here 60% of the total amount of volatile compounds were esters. Earlier studies were made on black currant buds (Rigaud et al., 1986; Nishimura and Mihara, 1988; Latrasse, 1991; Quere and Latrasse, 1990; Piriy et al., 1995) or homogenized black currant berries (Sydow and Karlsson, 1971a; Karlsson-Ekström and Sydow, 1973); in all of these cases the terpenoids dominated the aroma profile. This difference between the nectar and the buds or berries is probably due to the hydrophilic nature of the terpenoids, which are not soluble in water but are more inclined to adhere to fruit particles and are consequently removed from the nectar during the separation step. The nonvolatile terpinyl glucosides are
hydrophilic and will be extracted into the nectar, as are the short-chain esters.

Table 1 displays 98.3% of the total amount of volatiles trapped from the headspace of black currant nectar. The three types of nectars were compared by statistical analysis, and significant differences were found for 13 compounds (another 2 compounds were very close to the 5% level; Table 1). Ten of these compounds were identified, and all except one turned out to be esters; these esters were methyl butanoate, ethyl butanoate, methyl hexanoate, methyl heptanoate, ethyl heptanoate, hexyl acetate, ethyl decanoate, methyl decanoate, and 2-hexanone. The only exception was the terpene, α-caryophyllene. Eight of these esters decreased significantly in quantity as a result of the enzyme treatment. Terpinyl acetate apparently increased in concentration during pasteurization, but it could be that this compound is so labile that it is broken down by endogenous enzymes during aroma sampling of the untreated nectar; consequently, the highest concentration is found in the pasteurized nectar.

Black currant wine, juice, and juice concentrate from a commercial winery have been previously studied using dynamic headspace technique and column trapping (Leino and Kallio, 1993). The juice was fermented to black currant wine by the action of yeast enzymes; during this process ethyl hexanoate and ethyl octanoate increased considerably, while methyl octanoate, methyl...
decanoate, and bornyl acetate decreased significantly. Unfortunately, compounds that boil at lower temperatures were not collected because the column trapping was done at a rather high temperature (40 °C).

Enzyme treatment in apple juice production may cause negative sensory changes (Dür, 1981). After treatment of apple mash with 1% Pectinex super (Ferment AG, Basel) for 24 h at 22 °C, the residual concentrations of butyl acetate and hexyl acetate were only ≈1% for each (Dür, 1981). This is in good agreement with our study in which butyl acetate and hexyl acetate were among the eight esters that displayed significant breakdown.

The terpenes turned out to be quite resistant to enzyme treatment and pasteurization. Only 1 of the 14 identified terpenes or terpenoids changed significantly; this was α-caryophyllene (α-humulene), which decreased in concentration during enzyme treatment. The other identified terpenes and terpinols were α-pinene, β-pinene, 3-carene, α-phellandrene, myrcene, α-terpinene, limonen, eucalyptol, (Z)-ocimene, (E)-ocimene, β-caryophyllene, 4-terpineol, and α-terpinol. This result is in accordance with the findings of Leino (1993), who heated black currant nectar at 80 °C for 4 min and also found very small changes in the concentration of the terpenes and terpinols compared to the unheated nectar.

Pectinex Ultra SP (Novo Nordisk Ferment) is an industrial pectinase preparation derived from cultures of Aspergillus niger. After fermentation, the desired enzymes are recovered and purified. With the main pedolytic activities, the enzyme preparation will have hemicellulolytic and cellulolytic (β-glucosidase) activities (Canal-Llauberes, 1993). It might be expected that terpenoids would be liberated from their glucoside precursors by the action of β-glucosidase during the enzyme treatment, but this reaction does not occur in black currant nectar. The explanation is that β-glucosidase activity is totally inhibited by the glucose and fructose from the berries (Canal-Llauberes, 1993; Rosenberg, 1995).

The potency of different aroma compounds was estimated by a GC-sniffing evaluation (Table 1). The most significant contributors to the aroma profile were the esters methyl butanoate, ethyl butanoate, methyl decanoate, and ethyl hexanoate and the terpenes α-pinene, limonen, and β-phellandrene. In the case of (Z)-ocimene/ethyl hexanoate and nonanal/methyl octanoate, it cannot be certainly established which of the compounds is responsible for the fruity aroma. However, ethyl hexanoate has been described as powerful and fruity, and nonanal has been described as floral, citrus, and orange-like (Aldrich Chemical Co., 1996). Although the GC-sniff method excludes synergistic effects among the aroma compounds, it may be concluded that both the esters and the terpenoids are important to the aroma of black currant nectar.

Volatile compounds from black currant have been previously studied by different methods. Three different concentration procedures have been used: direct solvent extraction (Marriott, 1988), steam distillation (Latrasse et al., 1982), and dynamic headspace with column adsorption (Leino and Kallio, 1993). Marriott (1988) compared steam distillation and solvent extraction of black currant leaves; the recovery of volatile compounds differed greatly: some terpenoids were found in higher concentration in the steam distillate, and some terpenoids in higher concentration in the solvent extract. In our opinion this discrepancy could be caused either by chemical changes during steam distillation or by different solubilities among terpenoids in the chosen solvents. In the present study the dynamic headspace technique was applied. The headspace method was chosen because it is considered to be a gentle technique in which no heat is applied during aroma sampling, and this is very important, since heating could cause aroma changes that would be confounded with the effect of nectar pasteurization.

In the current study 4-methoxy-2-methyl-2-mercaptobutane was not identified, and the cat urine smell was not recognized by the GC-sniffing. This mercaptobutane was identified by Rigaud et al. (1986) after preconcentration of the volatiles of black currant bud oil by steam distillation, but it has not yet been identified in black currant juice or berries.

Extensive breakdown of C-6 aldehydes was found by Schreier et al. (1977) during enzyme treatment of red currant berries. In our study this seemed to be confirmed in the case of hexanal (P = 0.054), but not for the higher aldehydes.

No significant differences between nectars were found in the sensory evaluation of taste and odor. The differences between the three types of nectar were apparently too small to be recognized clearly by the judges, and random variation dominated the variation between the nectar types. The major reason for the lack of significance must be found in the fact that black currant nectar brings about a very aromatic effect to the organs of taste, and this makes the taste evaluation difficult.

From the present study, it can be concluded that enzyme treatment of black currant mash for 2 h at 50 °C causes major breakdown of the esters, which, together with some of the terpenes, are responsible for the fruity aroma notes of black currant nectar. The terpenes seem to be resistant to the enzymatic treatment. Pasteurization at 88 °C for 27 s did not cause any major change but, on the contrary, probably stabilized the aroma by inactivating the endogenous enzymes.

The best nectar production process is considered to be one that causes minimal changes to the original black currant berry aroma. From the present study it is clear that enzymatic activity is the main source of aroma alteration during processing. It was furthermore demonstrated that short-time heat treatment causes no significant changes to the black currant aroma content. On the basis of these findings the following recommendations for gentle black currant nectar processing can be made:

1. The enzyme treatment should be limited.
2. Processing should be continuous at moderate or low temperatures, i.e., 0–20 °C.
3. The heat treatment of the nectar should be brief.

LITERATURE CITED

Aldrich Chemical Co. Flavors and Fragrances; Aldrich: Milwaukee, WI, 1996.


Received for review June 9, 1997. Revised manuscript received December 1, 1997. Accepted December 7, 1997. This work was supported by the Danish Directorate for Development.