

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 terpenols 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). This characteristic is, to our knowledge, unique to black currant, and this means that the same terpenoids are 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). 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 differentiate 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 pectolytic 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 β -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).

* Author to whom correspondence should be addressed (telephone +45-6599 1766; fax +45-6599 1756; e-mail civ@alt.sp.dk).

[†] Danish Institute of Agricultural Sciences.

[‡] Present address: Blaesenborgvej 9, 4320 Lejre, Denmark.

[§] The Royal Veterinary and Agricultural University.

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 blended at 0 °C in a Fryma ML-250 and centrifuged at 20 °C in a screw-strain separator model Siebtechnik at 600g; for each 20 kg of raw nectar, 28 kg water was added, sugar concentration was adjusted to 14% w/w, and the nectar was frozen at -26 °C. Enzyme-treated samples were blended, enzyme treated, 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 siloxanized 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, 2.05 µg of 4-methyl-1-pentanol (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 cleaned with hot running tap water for 5 min followed 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 an HP-Innowax capillary column (60 m, 0.25 mm i.d., 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 200 °C 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 JEOL-JMS AX 505W (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 MS 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 nectars were evaluated at each session because the astringent effect of black currant nectar makes taste evaluation 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-Innowax column (HP part 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 GLM procedure was used for 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 (Leino 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; Piry 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 hydrophobic 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 terpenyl glucosides are

Table 1. Compounds Identified in Black Currant Nectar [Juice Was either Untreated (Control), Pasteurized, or Enzyme Treated]

compound (or main mass spectral ions)	control (ng/500 g)	pasteurized (ng/500 g)	enzyme treated (ng/500 g)	K_I^a	odor (GC-sniffing) ^b
ethyl acetate	1296 ± 113	1540 ± 224	1223 ± 186		
methyl butanoate	173488 ^d ± 3012	179514 ^d ± 26062	68267 ^e ± 10756	1001	fruit/orange/fruit chewing gum
α-pinene	392 ± 25	554 ± 67	593 ± 142	1022	sweet/fruitlike
ethyl butanoate	36634 ^d ± 1899	35441 ^d ± 4848	13069 ^e ± 2047	1048	fruit chewing gum/strawberry
methyl 3-butene-2-ol ^c	1422 ± 134	1575 ± 192	2378 ± 471	1051	
butyl acetate	101 ^d ± 14	108 ^d ± 6	42 ^e ± 1	1082	
hexanal (<i>P</i> = 0.054)	1729 ± 322	1838 ± 288	786 ± 131	1091	
49, 74, 43, 84, 57	119 ± 24	139 ± 18	64 ± 15	1098	
β-pinene	261 ± 7	320 ± 50	228 ± 60	1108	
methyl butenoate ^c	191 ^d ± 17	200 ^d ± 25	118 ^e ± 11	1118	
43, 55, 70, 93, 136 (monoterpene)	57 ± 2	74 ± 10	100 ± 27	1132	
spectrum identical with $K_I = 1132^*$	143 ± 11	147 ± 4	46 ± 8	1134	
3-carene	4494 ± 195	6355 ± 1073	6524 ± 1788	1152	
α-phellandrene	43 (<i>n</i> = 1)	87 ± 15	123 ± 29	1153	
myrcene	560 ± 27	686 ± 117	753 ± 193	1169	
119, 91, 77 (aromatic)	67 ± 6	114 ± 193	102 ± 12	1181	
α-terpinene	575 ± 26	986 ± 193	1163 ± 293	1183	
heptanal	60 ± 10	87 ± 11	50 ± 5	1194	
methyl hexanoate	14497 ^d ± 886	14026 ^d ± 2025	3897 ^e ± 593	1197	
limonene	2126 ± 340	2223 ± 392	3080 ± 913	1202	citrus fruit
β-phellandrene ^c	1109 ± 33	1362 ± 236	983 ± 235	1213	menthol
eucalyptol	207 ± 2	202 ± 16	218 ± 12	1216	
(<i>E</i>)-2-hexenal	55 ± 2	80 ± 19	49 ± 4	1232	green pepper/radish leaves
(<i>Z</i>)-ocimene	642 ± 42	891 ± 169	1038 ± 279	1242	
ethyl hexanoate	2436 ^d ± 190	2378 ^d ± 380	711 ^e ± 11	1244	fruity/orange/ strawberry
93, 91, 136, 121, 77 (monoterpene)	1440 ± 55	2454 ± 469	2528 ± 649	1252	
(<i>E</i>)-ocimene	445 ± 18	577 ± 144	576 ± 156	1259	
<i>p</i> -cymene	531 ± 100	728 ± 86	690 ± 79	1278	
hexyl acetate	123 ^d ± 8	135 ^d ± 19	71 ^e ± 14	1282	
93, 121, 136, 91, 77 (monoterpene)	1846 ± 50	2424 ± 463	2383 ± 604	1288	
octanal	59 ± 17	61 ± 10	32	1298	
57, 49, 41, 43, 71	54 ± 1	51 ± 5	39 ± 10	1300	
1-octen-3-one ^c	61 ± 3	90 ± 20	67 ± 8	1313	mushroom
(<i>E</i>)-2-heptenal*	39	76 ± 5	186 ± 8	1336	
2-heptenal (<i>E/Z</i>) ^c	169 ± 9	229 ± 42	171 ± 23	1339	
methyl octanoate	1641 ^d ± 106	1567 ^d ± 273	817 ^e ± 126	1398	
nonanal	101 ± 7	146 ± 46	79 ± 24	1403	acid/winegum/orange
ethyl octanoate (<i>P</i> = 0.056)	503 ± 30	589 ± 88	328 ± 48	1445	grass/green pepper
(<i>E</i>)-2-octenal	56 ± 5	87 ± 17	90 ± 11	1451	
unknown	57 ^d (<i>n</i> = 1)	65 ^d ± 3	39 ^e ± 3	1497	
bornyl acetate	28 (<i>n</i> = 1)	61 ± 5	42 ± 12	1594	
methyl decanoate ^c	349 ± 28	499 ± 104	326 ± 127	1604	flowers
β-caryophyllene	232 ± 4	525 ± 108	452 ± 107	1608	
4-terpineol	527 ± 28	718 ± 91	589 ± 51	1617	
methyl benzoate	236 ^d ± 19	318 ^d ± 33	180 ^e ± 9	1641	
ethyl decanoate ^c	222 ± 17	245 ± 19	195 ± 8	1645	
81, 67, 95, 43, 123	227 ± 17	364 ± 81	225 ± 49	1673	
ethyl benzoate	87 ± 3	166 ± 27	136 ± 36	1681	
α-caryophyllene	155 ^d ± 7	155 ^d ± 12	70 ^e ± 7	1687	
terpinyl acetate	75 ^d ± 5	110 ^e ± 13	62 ^d ± 7	1710	
α-terpineol	43 ± 8	69 ± 10	26 (<i>n</i> = 1)	1718	
unknown	56 ^d ± 3	97 ^e ± 14	56 ^d ± 3	1767	

^a K_I is Kovats linear retention indices. ^b GC-sniffing, the described odors were recognized at least twice as *strong* odors. Compounds were identified by mass spectrometry and verified by retention time and MS of reference compounds unless otherwise stated. ^c Tentatively identified by mass spectrometry. ^d ^e Samples are significantly different at 95% level (*P* < 0.05). An asterisk indicates nonsignificance because this compound is identical with adjoining compound. Statistical evaluation as described under Materials and Methods.

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; the esters were methyl butanoate, ethyl butanoate, butyl acetate, methyl hexanoate, methyl benzoate, ethyl hexanoate, hexyl acetate, methyl octanoate, and terpinyl acetate. 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 (Dürr, 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 (Dürr, 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 terpenols 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, limonene, eucalyptol, (*Z*)-ocimene, (*E*)-ocimene, β -caryophyllene, 4-terpineol, and α -terpineol. 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; besides the main pectolytic 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; Rogerson, 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, limonene, 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 (Marriot, 1988), steam distillation (Latrasse et al., 1982), and dynamic headspace with column adsorption (Leino and Kallio, 1993). Marriot (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 terpe-

noids 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 astringent 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.
- Canal-Llauberes, R. M. *Enzymes in Winemaking*. In *Wine Microbiology and Biotechnology*; Fleet, G. H., Ed.; Harwood Academic Publishers: Lausanne, Switzerland, 1993.
- Dürr, P.; Schobinger, U.; Zellweger, M. Das Aroma von Apfelmaishe bei deren Verflüssigung durch Pektinasen und Zellulasen. *Lebensm. Wiss. Technol.* **1981**, *14*, 268–272.

- Enevoldsen, K. In *Production of Fruit Flavour in Plant Tissue Culture*. Ph.D. Thesis, Biotechnology Research Division, Danisco A/S, Copenhagen, Denmark, 1991.
- Gueguen, Y.; Chemardin, P.; Janbon, G.; Arnaud, A.; Galzy, P. A Very Efficient β -glucosidase Catalyst for the Hydrolysis of Flavor Precursors of Wines and Fruit Juices. *J. Agric. Food Chem.* **1996**, *44*, 2336–2340.
- ISO 5496. Sensory analysis—Methodology—Initiation and training of assessors in the detection and recognition of odors. International Organization for Standardization, Genève, Switzerland, 1992.
- ISO 8586-1. Sensory analysis—General guidance for selection, training and monitoring of assessors. Part 1: Selected assessors. International Organisation for Standardization, Genève, Switzerland, 1993.
- Karlsson-Ekström, G.; Sydow, E. The Aroma of Black Currants. VII. The Influence of some Processing Parameters on the Aroma of Black Currants. *Lebensm. Wiss. Technol.* **1973**, *6*, 165–169.
- Latrasse, A. Fruits III. In *Volatile Compounds in Foods and Beverage*; Maarse H., Ed.; Dekker: New York, 1991.
- Latrasse, A.; Rigaud, J.; Sarris, J. L'Arôme du Cassis (*Ribes nigrum* L.) Odeur Principale et Notes Secondaires. *Sci. Aliments* **1982**, *2*, 145–162.
- Leino, M.; Kallio, H. Volatile compounds of blackcurrant juice and wine. *Z. Lebensm. Unters. Forsch.* **1993**, *196*, 410–414.
- Marriott, R. J. Isolation and Analysis of Blackcurrant (*Ribes nigrum*) leaf oil. In *Flavors and Fragrances: A World Perspective*; Proceedings of the 10th International Congress of Essential Oils, Washington DC, Nov 16–20, 1986; Lawrence, B. M., et al., Eds.; Elsevier Science Publishers: Amsterdam, 1988; pp 387–403.
- Nishimura, O.; Mihara, S. Aroma Constituents of Blackcurrant Buds (*Ribes nigrum*). In *Flavors and Fragrances: A World Perspective*; Proceedings of the 10th International Congress of Essential Oils, Washington DC, Nov 16–20, 1986; Lawrence, B. M., et al., Eds.; Elsevier Science Publishers: Amsterdam, 1988; pp 375–386.
- Piry, J.; Pribela, A.; Durcanská, J.; Farkas, P. Fractionation of volatiles from blackcurrant (*Ribes nigrum* L.) by different extractive methods. *Food Chem.* **1995**, *54*, 73–77.
- Quere, J. L. L.; Latrasse, A. Composition of the Essential Oils of Blackcurrant Buds (*Ribes nigrum* L.). *J. Agric. Food Chem.* **1990**, *38*, 3–10.
- Rigaud, J.; Étievant, P.; Henry, R.; Latrasse, A. Le Métoxy-4 Méthyl-2 Butanethiol-2, un Constituant Majeur de l'Arôme du Buorgeon de Cassis (*Ribes nigrum* L.). *Sci. Aliments* **1986**, *6*, 213–220.
- Rogerson, F.; Grande, H.; Silva, M. Enzymatic enhancement of the free monoterpenol content of a Portuguese wine from a single, native grape variety; "Trajadura". *Biotechnol. Lett.* **1995**, *17*, 35–40.
- Schreier, P.; Drawert, F.; Junker, A. Über die quantitative Zusammensetzung natürlicher und technologisch veränderter Aromen. III Veränderungen und Neubildungen von Aromastoffen bei der Herstellung von Säften aus roten Johannisbeeren. *Lebensm. Wiss. Technol.* **1977**, *10*, 377–340.
- Schwimmer, S. Enzymatic aroma genesis in fruits, flavorants and beverages. In *Source Book of Food Enzymology*; Schwimmer, S., Ed.; AVI Publishing: Westport, CT, 1981; pp 388–405.
- Stone, H. Quantitative Descriptive Analysis (QDA). In *Manual on Descriptive Analysis Testing for Sensory Evaluation*; Hootman, R., Ed.; ASTM: Philadelphia, PA, 1992.
- Sydow, E.; Karlsson, G. The Aroma of Black Currants. IV. The influence of heat measured by instrumental methods. *Lebens. Wiss. Technol.* **1971a**, *4*, 54–58.
- Sydow, E.; Karlsson, G. The Aroma of Black Currants. V. The Influence of Heat Measured by Odour Quality Assessment Techniques. *Lebensm. Wiss. Technol.* **1971b**, *4*, 152–157.

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.

JF970513Y