AROMA
OF BLACK CURRANT JUICE

The effect of thermal treatment and juice concentration

PH.D. THESIS BY CAMILLA VARMING | 2005

THE ROYAL VETERINARY AND AGRICULTURAL UNIVERSITY
DEPARTMENT OF FOOD SCIENCE
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Ph.D. Thesis by
Camilla Varming
Cand.techn.al.

Quality and Technology
Department of Food Science
The Royal Veterinary and Agricultural University
Rolighedsvej 30, 1958 Frederiksberg C., Denmark

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Preface

This thesis represents research work to fulfil the requirements for a Ph.D. degree at the Royal Veterinary and Agricultural University, Denmark (KVL). The Ph.D. study has been a part of the project "Sour cherry and black currant - optimizing juice quality". The research work was financially supported by the Ministry of Food, Agriculture and Fisheries via the Danish Vertical Fruit and Vegetable Network. The project was collaboration between the Technical University of Denmark, Vallø Saft A/S, Bioscan A/S - later University of Southern Denmark, and FLS Miljø A/S. My employment on the project began as research assistant, and was developed into a Ph.D. study over a subsequent period of one year and nine months. The principal supervisor was Associate Professor Leif Poll, KVL and co-supervisor was Associate Professor Mogens Larsen Andersen, KVL.

I would like to acknowledge my supervisors for their engagement and inspiring discussions. Special thanks to Leif Poll for encouragement, and always taking his time for debate; and to Mogens Larsen Andersen for Food Chemistry guidance and a problem-solving attitude. I would like to thank the laboratory technicians Mehdi Farahani and Karina Fife for the conscientious work carried out in relation to my project, my office-mate Ghita Nielsen for her assistance and essential discussions, and the remainder of the Quality and Technology group at KVL for creating a good working environment. I am grateful to Morten Friis, Rico Bagger-Jørgensen, and Detlef Ulrich; as well as to Derek V. Byrne for proof reading of this manuscript. Finally, I am grateful to Peter, Albert & Felix for their patience and support during this work.

October 2005

Camilla Varming

Summary

Industrial production of black currant juice involves several processing steps and is most often finished by concentration, in order to reduce volume and stabilize the juice for storage and transport. Heat-induced evaporation of water, associated with the processes of aroma recovery and juice concentration, is the conventional method of concentrating fruit juices. In the present Ph.D. thesis are issues connected to the influence of thermal treatment and concentration on black currant juice aroma, in addition to aspects associated with methods for analysis of the aroma compounds investigated.

The influence of thermal treatment on black currant juice aroma was studied in temperature and time ranges relevant for black currant juice concentration processes. Heating at 90°C affected the aroma and sensory characteristics of the juice, by increasing levels of aldehydes, furans, phenols, and benzene derivatives, but slightly decreasing levels of most esters and alcohols. The concentration of the majority of terpenes increased, whereas some terpene alcohols decreased.

The importance of acid catalyzed reactions as the cause of the changes observed in the terpene complex during thermal treatment of black currant juice was evaluated. Results showed that the selected individual terpenes were degraded and converted into other terpene hydrocarbons and oxygenated terpenes. By the use of β-glycosidase, applied in two different manners, a range of alcohols and oxygenated terpenes of the juice were demonstrated to be present as bound to glycosides. Hence, it was concluded that the changes in terpene concentrations during thermal processing might be a combination of acid-catalyzed reactions, and to a lesser degree release from a pool of glycosidically bound terpenes.

Aroma changes during industrial scale concentration of black currant juice were investigated by determination of aroma recovery in aroma distillate, juice concentrate and singlings. Relative to the initial base juice significant reductions were seen for the majority of the compounds, including alcohols, esters and most terpenes. On the contrary, an increase of furfural, benzene derivatives and some carbonyls was seen. Esters and oxygenated terpenes are important to black currant odour, hence the changes are expected to influence the sensory quality of the reconstituted juice by a decrease in fruity notes. The increase of the aroma compounds observed during juice concentration can be explained by the thermally induced changes demonstrated. However, the observed reductions are only to a limited extent explained by the influence of heat, suggesting that aroma changes during juice concentration are not only thermally induced but are also influenced by other processing factors.

Membrane distillation techniques operating at lower temperatures have been introduced in concentration of fruit juices. Vacuum membrane distillation and sweeping gas membrane distillation were evaluated with respect to their overall aroma recovery performance, and these methods were found to be superior to the conventional evaporation concentration. However, problems remaining to be solved are, that a higher °Brix of the juice concentrate than obtained is required as well as the aroma distillate needs further concentration.

Considering methods for analysis of the aroma compounds, properties of the adsorbent materials Tenax TA and GR used for headspace collection of volatiles, were studied. It was demonstrated that black currant juice terpene alcohols were subject to acid catalysed degradation on Tenax GR during purge and trap sampling.

Compounds important for the aroma of black currant juice as determined by the nasal impact frequency method were applied to aroma isolates obtained by different isolation techniques, including solvent extraction and three headspace methods. The method of aroma isolation was found to influence the results of gas chromatography-olfactometry, by the number and type of identified aroma compounds as well as by order of their perceived importance.

Resumé

Industriel fremstilling af solbærsaft involverer adskillige procestrin og afsluttes oftest med opkoncentrering for at reducere volumen og stabilisere saften med henblik på lagring og transport. Varmeinduceret afdampning af vand, associeret med aromagenindvinding og saftkoncentrering, er den konventionelle metode, som anvendes ved opkoncentrering af frugtsaft. I nærværende Ph.D. afhandling er aspekter, forbundet med indflydelsen af varmebehandling og opkoncentrering på aromastoffer i solbærsaft, samt emner relateret til aromaanalysemetoder, undersøgt.

Indflydelsen af varmebehandling på solbærsaftaroma blev undersøgt i temperatur– og tidsområder, relevante for solbærsaftens opkoncentreringsprocesser. Opvarmning ved 90°C påvirkede saftens aromamæssige og sensoriske egenskaber ved en stigning i indhold af aldehyder, furaner, fenoler og benzenderivater, men medførte en mindre reduktion af de fleste estre og alkoholer. Koncentrationen af de fleste terpener steg, men for nogle terpenalkoholer faldt den.

Betydningen af syrekatalyserede omlejringer som årsag til ændringerne, observeret i terpenkomplekset ved varmebehandling af solbærsaft, blev efterprøvet. Resultaterne viste at de undersøgte terpener blev nedbrudt og omdannet til andre terpen-hydrocarboner og oxygenerede terpener. Ved brug af β-glycosidase, anvendt på to forskellige måder, blev det desuden vist at en række alkoholer og oxygenerede terpener fandtes i saften som glykosidbundne. Det konkluderedes at ændringerne i terpenkoncentrationer, ved opvarmning, kan skyldes en kombination af syrekatalyserede reaktioner og, i mindre grad, frigørelse af glykosidbundne terpener.

Aromaændringer ved industri-skala opkoncentrering af solbærsaft blev undersøgt ved bestemmelse af aromagenfinding i aromadestillat, saftkoncentrat og luttervand. I forhold til basissaften blev signifikante tab set for de fleste stoffer, inklusiv alkoholer, estre og en stor del af terpenerne. Modsat sås en stigning af furfural, benzenderivater og visse carbonylforbindelser. Estre og oxygenerede terpener har betydning for solbærlugt, så ændringer må forventes at påvirke den sensoriske kvalitet af den genfortyndede saft i form af mindre grad af frugtagtig lugt. Stigningen i aromaforbindelser, observeret ved opkoncentrering af saft, kan forklares ved de påviste varmeinducerede ændringer. Imidlertid kan de observerede tab kun i begrænset omfang forklares med varmepåvirkning, hvilket tyder på at aromaændringer ved opkoncentrering ikke kun er forårsaget af varme, men også er influeret af andre procesfaktorer.

Membrandestillationsteknikker, der opererer ved lavere temperaturer, er lanceret til opkoncentrering af frugtsaft. Vakuummembrandestillation og sweeping gas membrandestillation blev vurderet i forhold til den samlede aromagenfinding ved processerne, og disse metoder viste sig at være bedre end konventionel opkoncentrering. Imidlertid er der problemer som mangler at blive løst, idet en højere °Brix af saftkoncentratet end den opnåede er nødvendig, ligesom aromadestillatet må opkoncentreres yderligere.

Med hensyn til aromaanalysemetoder undersøgtes egenskaberne af fældematerialerne Tenax TA og GR, der anvendes til headspaceopsamling af aromastoffer. Det viste sig at terpenalkoholerne i solbærsaft var genstand for syrekatalyseret nedbrydning på Tenax GR i forbindelse med headspaceopsamlingen.

Stoffer der har betydning for aroma af solbærsaft blev bestemt ved "nasal impact frequency" metoden, som blev anvendt på aromaisolater fra forskellige isolationsteknikker, herunder solventekstraktion og headspacemetoder. Aromaisolationsmetoden viste sig at have indflydelse på gaskromatografi-olfaktometri-resultaterne ved antal og type, samt rangordning af de identificerede aromastoffers opfattede betydning.

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List of publications

Paper I
Varming, C. and Poll, L. Aroma recovery during concentration of black currant juice. In: Flavour Research at the Dawn of the Twenty-First Century. Proceedings of the 10th Weurman Flavour Research Symposium. Le Quéré, J. L., Etiévant, P. X. (Eds.); Lavoisier: Cachan, France, **2003**, pp 741-744.

Paper II
Varming, C., Petersen, M. A. and Poll, L. Comparison of isolation methods for the determination of important aroma compounds in black currant (*Ribes nigrum* L.) juice, using NIF profiling. *Journal of Agricultural and Food Chemistry*, **2004**, 52, 1647-1652.

Paper III
Bagger-Jørgensen, R., Meyer, A. S., Varming, C. and Jonsson, G. Recovery of volatile aroma compounds from black currant juice using vacuum membrane distillation. *Journal of Food Engineering,* **2004**, 64, 23-31.

Paper IV
Varming, C., Andersen, M. L. and Poll, L. Influence of thermal treatment on black currant juice (*Ribes nigrum* L.) aroma. *Journal of Agricultural and Food Chemistry*, **2004**, 52, 7628-7636.

Paper V
Varming, C., Andersen, M. L. and Poll, L. Comparison of Tenax TA and Tenax GR as adsorbent materials for headspace sampling of black currant juice aroma. In: State-of-the–Art in Flavour Chemistry and Biology. Proceedings of the 7th Wartburg Symposium on Flavour Chemistry and Biology. Hofmann, T; Rothe, M. and Schieberle, P. (Eds.); Deutsche Forschungsanstalt für Lebensmittelchemie: Garching, Germany, **2005**, pp 373-377.

Paper VI
Bagger-Jørgensen, R., Meyer, A. S., Varming, C. and Jonsson, G. Recovery of volatile fruit juice aroma compounds by sweeping gas membrane distillation. Submitted to *Journal of Food Engineering*.

Paper VII
Varming, C., Andersen, M. L. and Poll, L. Monoterpenes in black currant (*Ribes nigrum* L.) juice: Role of heat induced changes and glycosidically bound terpenes. Submitted to *Journal of Agricultural and Food Chemistry*.

Paper VIII
Varming, C., Andersen, M. L. and Poll, L. Glycosidically bound alcohols of blackcurrant juice. Accepted for publication in Proceedings of the 11th Weurman Flavour Research Symposium.

Abbreviations

AEDA	aroma extract dilution analysis
AIJN	Association of the industry of juices and nectars from fruits and vegetables of the European Union
aw	water activity
CF	concentration factors
Charm	combined hedonic and response measurement
CV	coefficient of variation
EU	European Union
FID	flame ionization detection
GC	gas chromatograph
GC-MS	GC-mass spectrometry
GC-O	GC-olfactometry
GMP	good manufacturing practice
HPLC	high performance liquid chromatography
MD	membrane distillation
NIF	nasal impact frequency
P&T	purge and trap
P&T 15	15 minutes purge time
P&T 60	60 minutes purge time
RI	retention index
RO	reverse osmosis
SDE	simultaneous distillation extraction
SGMD	sweeping gas membrane distillation
SNIF	surface of nasal impact frequency
SPME	solid phase micro extraction
VMD	vacuum membrane distillation

1. Introduction

Currants were first cultivated in Europe in the fifteenth century and used mainly for medical purposes. Processing of currants and related berries began in the late 1800s and developed rapidly in the 1950-1960s. Currants grow in the form of scrubs, which are cultivated in temperate and cold regions of the northern and southern hemispheres (1). The world annual (2003) production of currants is 679.000 MT. Currants are mainly grown in Europe with Russia, Poland and Germany being the biggest producers, and currant is the most widely cultivated bush fruit in Europe (2).

Currants, belonging to the Saxifragaceae family, may be classified into black currants (*Ribes nigrum* L), red currants, and white currants (1). The colour of black currants is derived from anthocyanidins (1,3). The fruit is nutritional valuable with one of the highest levels of vitamin C of any fruits (3), and black currant is a good source of phenolic compounds with a range of antioxidant abilities (4).

Black currants have a characteristic flavour and aroma that distinguish them as value-added foods for preparing fruit products. Most of the crop is used for juice production (5) in addition to syrup, jam, wine, bakery goods, frozen products and essence; only a minor part of the fruit proportion is freshly consumed (1,3).

Overall, fruit juice and nectars constitutes 8% of the global soft drink sector. Fruit juices have gained volume sales and generally health issues are influencing the dynamics of the soft drink industry (6).

Industrial production of black currant berries to juice involves enzyme treatment, pressing, clarification and filtration as well as several heating steps. Concentration of fruit juices is most often applied as a final step in order to reduce volume and stabilize the juice for storage and transport. The conventional method of concentrating fruit juices is by heat induced evaporation of water, and recovery of the aroma compounds in a distillate that is subsequently added to the concentrate for reconstitution. During this process some thermally induced changes occur affecting the flavour quality of the reconstituted juice, hence the aroma of concentrated juice differs from that of fresh black currant juice (7). Recently, more gentle membrane process technologies for distillation and concentration of fruit juices have been introduced.

The primary aims of the present research project were to:

- investigate the aroma changes derived from thermal treatment of the juice
- examine the impact of concentration of black currant juice on its aroma and in part compare it to membrane distillation technology

Considering these aims, issues related to the analysis of aroma compounds in black currant juice occurred; hence secondary aims of the project were to:

- evaluate aspects associated with analysis of black currant juice aroma, that is adsorbent materials for headspace sampling, and the influence of isolation method on gas chromatography-olfactometry results.

2. Black currant juice production

Black currant is primary produced for industrial processing, and together with sour cherry and strawberry it is the most important berry crop in Denmark (8). Botanically the black currant fruit is a true berry, with the seeds enclosed in a fleshy pericarp (9). The soft and small berries are mechanically harvested at a stage of optimum maturity, and they are highly sensitive, especially to mechanical injuries, requiring care during transport and subsequent fast processing, as injuries lead to deterioration (3). The black currant berry undergoes several steps in the juice process, of which it shares common features with other soft fruits (1). The concentration of fruit juices can be considered a final processing step for many fruits (10).

2.1 Composition of black currant berry

The black currant fruit is nutritional valuable containing large amounts of fructose, glucose, organic acids, and pectins, and it is particularly rich in ascorbic acid (Table 1). Black currant berries contains phenolic compounds such as phenolic acids (11) and flavonols (1,12); and the colour of black currants is derived from anthocyanidins, mainly cyanidin-3-glycoside, cyanidin-3-rutinoside, delphinidin-3-glycoside and delphinidin-3-rutinoside (13).

Table 1. Chemical composition of black currant berry and juice (14)[a].

Nutrient	Berry g/100g	Juice g/100g
Water	79	88
Ash	0.9	0.2
Protein	1.5	0.5
Lipids	1.3	0.5
Carbohydrate total	17.3	11
Fructose	5.0	- [b]
Glucose	4.2	-
Sucrose	0.5	-
Fiber	5.8	-
Citric acid	2.4 (12)	-
Malic acid	0.2 (12)	-
Ascorbic acid	0.18	0.066
Pectin	1.1 (15)	0.25[c] (12)

[a] Most figures are given as average. [b] Not reported. [c] Determined as galacturonic acid.

There are on-going breeding programmes in the leading black currant-producing countries, emphasising yield, stability of cropping and resistance to pests and diseases (16). Processing companies place emphasis on juice quality indicators such as colour and sugar content. Significant differences between varieties exist in fructose, glucose, titratable acids, malic acid,

ascorbic acid and colour (17). The cultivar Ben Lomond[1] used for juice production in the present project is the most widely used cultivar in Denmark (8). Compared to nine other varieties it is high in ascorbic acid, low in total sugars, high in titratable acids, and low in pH (17).

2.2 Juice processing

Vallø Saft A/S, Denmark, provided the juice samples in the present project (harvest years 1999 and 2003) for the experiments referred to in paper I-VIII. Vallø Saft A/S has a considerable industrial production of black currant and other fruit juices. In the following, the general practices of soft fruit juice production are described after (1,3) and summarised together with procedures specifically applied in black currant juice processing at Vallø Saft A/S (18) (Figure 1). The figures of Figure 1 are specific for the production at Vallø Saft A/S, but practise vary between production sites.

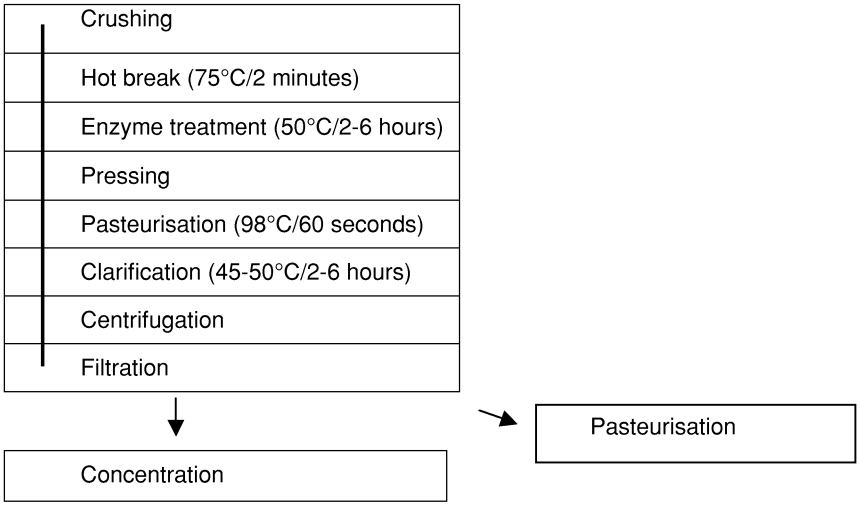

Figure 1. Schematic presentation of the black currant juice production process. General practices of soft fruit juice production summarised together with procedures at Vallø Saft A/S.

Fresh or frozen (thawed) fruits are used in juice production. The fruit is disintegrated for size reduction to aid in yield recovery and efficient extraction of pigments and other soluble components.

Heat (a hot break) is applied for cell rupture facilitating the extraction of cell juice, as well as for denaturation of enzymes (peroxidase and polyphenoloxidase) which would cause stability problems in clear juices at later stages of production.

Pectolytic enzyme treatment break down the cellular structure of the fruit making it possible for more juice to pass out of the fruit, and prevent pectin polymers forming gels.

[1] In the papers (I-VIII) incorrectly referred to as Ben Lemond.

The juice is obtained by pressing, and the press cake is sometimes extracted with water to obtain a greater juice yield.

Clarification is carried out with the aid of gelatine, bentonit and kiselsol for sedimentation of clouding components like protein, polyphenols and pectin. Enzymes (pectinease) can be added to help produce clear juices. By centrifugation the clouding components are removed.

The juice is pre-filtered on a vacuum filter and then ultra- or pressure filtrated. If not subject to a concentration step, the juice is then pasteurised and transferred to storage vessels and stored at 10°C. The yield of black currant juice production is approx. 90%.

Final °Brix (see definition in Section 2.2.1) of the black currant juice, referred to as single strength juice, is typically about 12 °Brix. At this stage the juice has a very acidic taste and therefore has to be sweetened.

Clear juice, per definition, has to be free of suspended particles and any other clouding components. Nectars or pulpy juices on the other hand contain certain amounts of suspended particles, of which pectin is the most important. Usually, a certain amount of sweetener e.g. sugar syrup, fruit acids (citric acid) and stabilizers are added to make nectars. Berry fruit juices are often mixed with juices from other kind of fruits, especially those with a less marked aroma; or used as ingredients in food and beverage production (1).

Fruit juices and nectars represent the largest volume of non-carbonated beverages that are sold in almost every market place. Fruit juice can be prepared as dilutable products containing a minimum of juice/fruit, added sugars/sweeteners, and possible additives (aroma/flavour, preservatives and colour), diluted by the consumer as a convenient means of producing soft drinks on the consumers' premises; or as ready-to-drink pre-packed beverages of which formulations are similar to dilutable beverage except dilution takes place at the manufacturer's premises (19). "The Danish ministry of family and consumer affairs" executive order of fruit juices etc laid down the following trade descriptions (20):

1) a: fruit juice (fermentable, but non-fermented product produced from sound, mature fruits possessing the colour, aroma and taste qualities characteristic for juice of the fruits from which it originate)
   b: reconstituted fruit juice made from concentrate
2) dilutable juice
3) dehydrated juice in the form of powder
4) nectar (composed of a minimum content of 1, 2 or 3; added sugar and water only)[2]
5) cider

[2] The definition of nectar in this regulative differs from that described in (1) above.

2.2.1 Quality issues and legislative concerns

The soluble solid content and titratable acidity are the major indicators to be taken into account when identifying the status and suitability of a juice product. Soluble solid relate directly to the sugars and fruit acids as these are the main contributors, and there is a direct relationship to the specific gravity of the solution as measured by °Brix. These parameters are used to evaluate maturity stage of the berries. Other quality considerations are lactic acid and ethanol concentrations as indicators of fermenting, as well as colour measurements. In addition there is legislation regarding pesticides, heavy metals and mycotoxins; and practically all customers demand microbial count of yeast and moulds. Quality criteria for berry on receipt of production site are judgement of maturity level of samples measured by °Brix and acid content as well as visual evaluation (18,21).

Processing quality criteria for juice within the European Union (EU) follows Code of Practice by the Association of the Industry of Juices and Nectars from Fruits and Vegetables of the EU (AIJN). This body has issued guidelines detailing standards for the range of juice products manufactured in the EU, and also on issues of good manufacturing practice (GMP) (18,21).

2.3 Juice concentration

2.3.1 Introduction and background

Fruit juice concentration is a separation procedure, of which the main purpose is to increase the content of dry solids (up to 65-70%) in the juice through water separation (10).

The first fruit juice concentrates produced through evaporation date from the beginning of the 1920ies. During this evaporation process the volatiles were lost together with water, and due to relatively high evaporation temperatures together with long residence times of the juice in the evaporator, the concentrates obtained were characterized by major chemical and sensory changes i.e. generation of cooked flavour and loss of fruit character and freshness (10). The so called cut back technique was applied to some fruit juices, by concentrating juice, and then mixing it with fresh juice to compensate the loss of flavour during concentration (22,23). In the 1940ies a procedure was developed for aroma recovery, and today's fruit juices are mostly concentrated using multi-stage vacuum evaporators at the lowest possible temperature with short residence time (10). Still though, concentration by this technique causes to some extend oxidation of compounds in the juice and loss of volatile compounds. Moreover, the process is high energy-intensive (23).

Juice concentration in multi-stage vacuum evaporators is associated with the processes of aroma recovery and aroma concentration. The volatile aroma compounds recovered are also known as essence (1). It is important that a maximum of the characteristic aroma components of the fruit is separated during aroma recovery (3), and also colour and vitamins shall be maintained as far as possible whereas undesired chemical processes shall be avoided (10). Aroma compounds removed during concentration are returned and water added through reconstitution when reproducing the diluted juice (1). The removal of a large fraction of water from the single

strength juice will reduce both weight and volume of products and result in cost savings of storage and transport. At the same time there are benefits over single strength juice in that the high concentration of dissolved solids reduces water activity (aw) inhibiting the growth of micro-organisms, there by improve stability and extend shelf life (21).

Alternative concentration procedures for fruit juices have found commercial application, but are not widely used (24). Freeze concentration maintain the aroma but are limited by a final concentration level of only 45-50% solids and high energy costs (1,10,23). Application of membrane processes is an emerging methodology to concentrate fruit juices requiring less energy and providing gentle treatment for retaining flavour and aroma (25). Reverse osmosis (RO) is a membrane concentration technique applied for pre-concentration of fruit juices, but it is limited by inability to reach the desired concentration level (1,25). Membrane distillation is a more recent development, and this issue in the form of vacuum membrane distillation and sweeping gas membrane distillation shall be touched on in Section 2.3.3; but discussion of matters except aroma recovery concerns is beyond the scope of this thesis. The reader is referred to (26) for further information on membrane distillation.

2.3.2 Conventional evaporation concentration

Fractional distillation of fruit juice in a two-step vacuum evaporator is illustrated in Figure 2. The quality of aroma concentrates is affected by the process parameters such as pressure, reflux ratio and temperature. During falling film evaporation concentration processes temperatures of 90-105°C, driven with heating steam, are applied with a residence time of approximately 5-10 minutes (10,18). In the concentration procedure dry matter of the juice concentrate is increased to 65-75%. The microbiological stability of fruit juice concentrates is largely influenced by their low pH-value (2.0-4.0) and the low water activity (aw 0.75-0.9) (10). Heating steam and cooling water requirements are the major factors determining operation cost (10,18).

Vacuum evaporation followed by distillation is the most common technique used for aroma recovery. Other processes for aroma recovery may be divided into the following groups: atmospheric operations, aroma recovery by stripping, and aroma recovery by liquid extraction (10,23,27,28). When evaporation takes place to concentrate fruit juice, volatile aroma substances are removed with the water vapours and subjected to fractional distillation and then concentrated to enrich the aroma compounds in the form of a clear liquid (10,24,27). The aroma is concentrated, typically to 1/100 - 1/200 the volume of the original food (23,27,28). Storability of the aroma concentrate is affected by the presence of an oil phase, oxygen and the storage temperature (10,28).

In a distillation process carried out under vacuum highly volatile aroma compounds may be lost through the vacuum pump. In addition to technological influencing factors also chemical changes are playing a role. Many of the components of the juice (Table 1, p. 3) may react with each other during the concentration process; in so doing high evaporation temperatures may especially lead to undesired colour and flavour changes as well as vitamin losses (10,22).

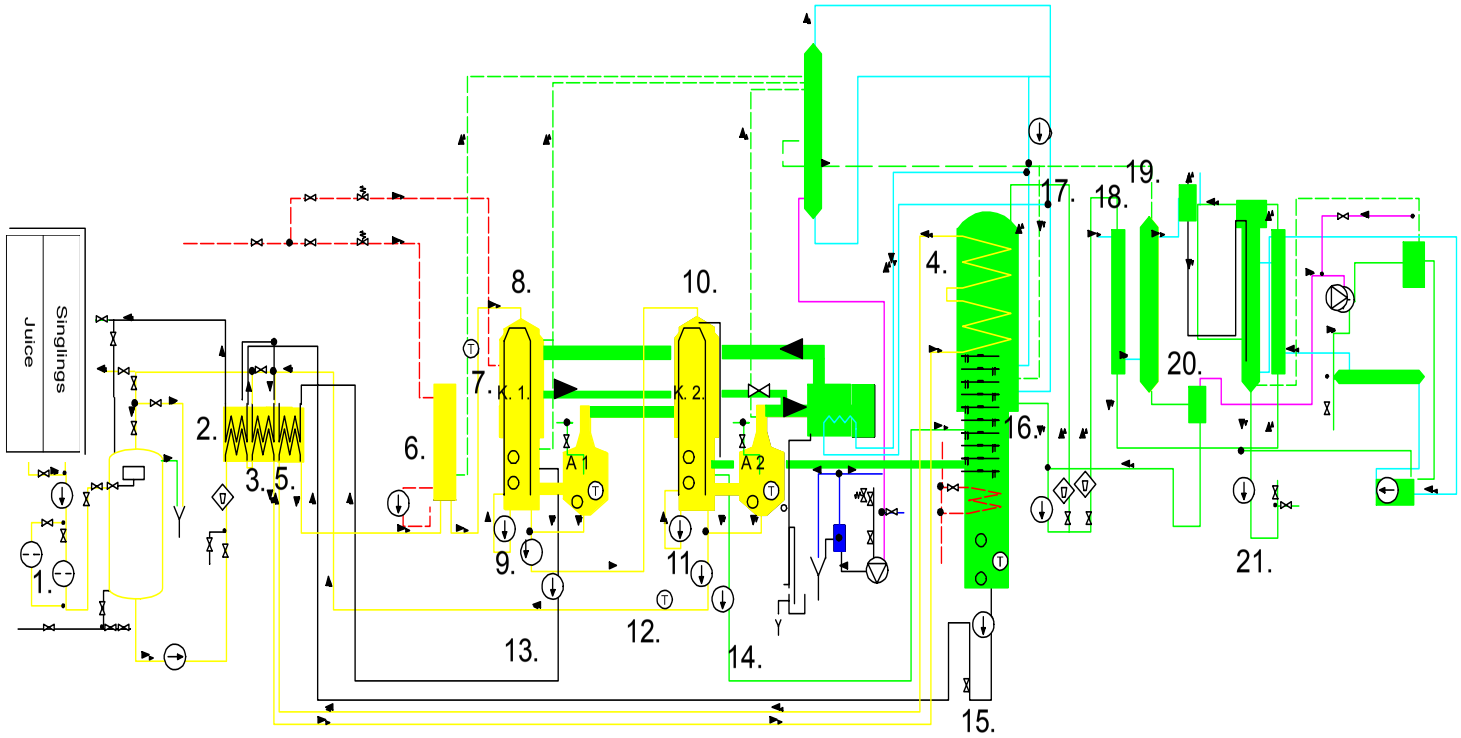

Figure 2. Schematic view of a 2-step combined evaporation and aroma recovery plant. 1 = pre-filtration of juice, 2-7 = heating steps, 8-11 = evaporation steps, 12 = juice concentrate (for further concentration) 13-15 = singlings, 16 = aroma tap, 17 = aroma circulation, 18 = hot aroma, 19 = aroma in the form of vapour, 20 = aroma recirculation, 21 = cooled aroma distillate for storage. Colour codes = Juice area; Aroma line; Singlings; Vacuum line; Cooling (*29*).

Because of the complexity of fruit aroma volatiles they behave differently during evaporation (27). For each type of fruit juice a specific degree of fruit juice evaporation is required. Considered the volatility of fruit juice aromas under vacuum, the percentage of juice evaporation required for aroma recovery is 10-50% of the amount of fruit juice, although higher evaporation rates might be necessary to separate the totality of aroma substances. Highly volatile compounds are completely removed through evaporation, whereas poorly volatile aromas are only volatilised in proportion to the quantity of water evaporated and to a certain extend are even retained (3,10,22). Black currant juice aroma compounds are highly to medium volatile i.e. they are almost completely evaporated at a degree of juice evaporation of 50% (Figure 3) (10).

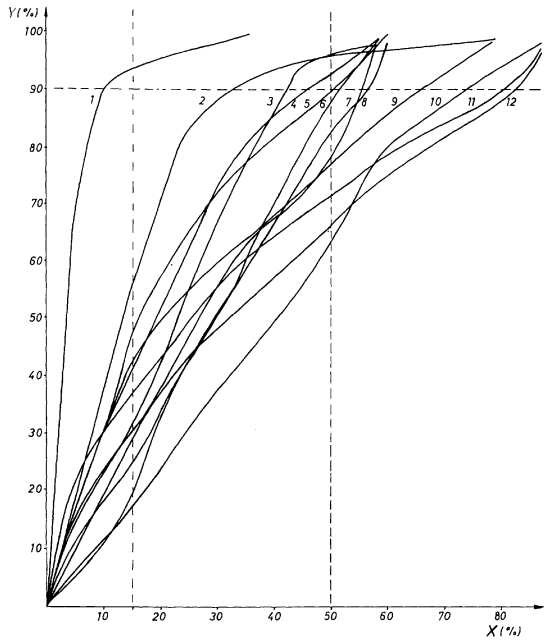

Figure 3. Dynamics of total aroma separation of fruit juices (*10*). Y in % = separation (evaporation) of total aroma in %, X in % = degere of juice evaporation; curves of aroma separation from juices: 1 = apple, 2 = plum, 3 = grape, 4 = black currant, 5 = pear, 6 = cherry, 7 = apricot, 8 = quince, 9 = peach, 10 = raspberry, 11 = blackberry, 12 = strawberry.

At Vallø Saft A/S the evaporation system (Unipektin) consist of two falling film evaporator units, namely a 2-step evaporator (Figure 2) and a 4-step evaporator (employed in paper I). The 2-step evaporator concentrates the juice from 12-24 °Brix, and the aroma is separated in this step, constituting 0.6% of the initial base juice. Reduced pressure is obtained from a vacuum blower, from which the outlet contains a minor unknown amount of aroma, as the siphon trap is not easily accessible. The run through time is approximately 4 minutes in the temperature range 80-92°C. The temperature of the aroma tower is about 70°C. In the 4-step evaporator the juice is further concentrated from 24 to 65 °Brix. The run through time is approximately 10 minutes with holding temperatures from 70 to maximum 107°C. The "singlings", constituting condensed evaporated water of the evaporator units, theoretically devoid of aroma, is discharged. Juice

concentrate and aroma distillate is separately stored in containers at 10°C. Accurate measures of the evaporation steps are difficult to obtain as measurements could be obtained on accessible parts only, and detailed engineering drawings are not released by the manufacturer (18,21).

2.3.3 Membrane distillation

Membrane distillation (MD) is a relatively new evaporative process in which two solutions at different temperatures are separated by a micro porous hydrophobic membrane into a retentate and a permeate (Figure 4). The driving force of the process corresponds to the partial pressure gradient and thus the thermal gradient between the two membrane sides. The driving force generates a flux from the warm side to the cold side. The phenomenon can be described as a three phase sequence: *(1)* Formation of vapour at the warm solution-membrane interface; *(2)* transport of aroma containing vapour through the micro porous system; *(3)* condensation of vapour at the cold side membrane-solution interface. Non-volatile compounds such as sugars dissolved in aqueous solutions can be completely retained and concentrated. The MD process can be carried out with different configurations, depending on the driving force by which the vapour is recovered from the membrane pores (25,30-32). The two MD methods assessed are vacuum membrane distillation (VMD) in paper III and sweeping gas membrane distillation (SGMD) in paper VI, both examined at feed temperatures of 10-45 ^{o}C.

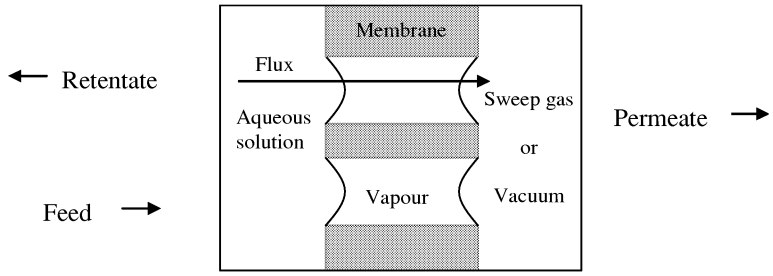

Figure 4. Schematic presentation of membrane distillation configurations (30).

Because membrane distillation can be carried out at temperatures which can be much lower than the boiling point of the solution, it can be used to concentrate produces sensitive to higher temperatures, like fruit juices (25,33). The potential advantages of membrane concentration techniques over conventional evaporation for concentrating fruit juices include improved product quality i.e. lower thermal damage to the product as well as lower energy consumption and equipment costs. One main disadvantage of MD processes has been the inability to reach the concentration of standard products as produced by evaporation. Recently, technological advances related to the development of new membranes and improvements in process engineering have been proved to overcome this limitation (25,34). VMD and SGMD are employed at pilot plant scale, but industrial scale production has not been reported (26).

In VMD (paper III) the membrane separates the liquid feed from a downstream gaseous phase and the mass transfer through the membrane is improved by applying a vacuum on the permeate side (Figure 4). Permeate condensation takes place outside the module, inside a condenser or a trap containing liquid nitrogen (laboratory scale). The process is characterised by evaporation of the liquid mixture at the liquid-membrane interface and by mass transport of vapours through the membrane pores. As a consequence, non-volatile compounds such as sugars dissolved in aqueous solutions can be completely retained and concentrated to high concentration values e.g. 50 °Brix at 60°C (30,32). The VMD technique creates the highest partial pressure gradients and thus higher fluxes and plant productivity compared to other MD techniques (30).

In SGMD (paper VI) an inert carrier gas circulates downstream the membrane surface flushing and collecting the permeated vapours (Figure 4). Permeate condensation occurs outside the module in an external condenser. Less work has been done in the area of SGMD probably because none of the variables remain constant along the module. The temperature, concentration, and the heat and mass transfers rates all change within the module during the progression of the sweeping gas (35-37).

3. Methods of aroma determination in black currant juice

Aroma compounds in foods are present in very low concentrations (ppt-ppm range) challenging their isolation, concentration and detection. In the aroma isolation step special attention must be paid with respect to potential destruction of aroma components and formation of artefacts (38). The two most common methods for isolation of volatiles are headspace methods and extraction (39). Solid phase microextraction (SPME), a more recently developed technique comprising thin polymeric films coated onto fused silica fibers (40), is used in (41,42) for the isolation of black currant berry volatiles.

As the black currant juice in this study is pasteurised it is not subject to microbiologically and enzymatically induced aroma changes during isolation of the volatiles. Foaming, or formation of emulsions is not a problem due to the low protein and fat content, and the liquid homogeneous nature makes it simpler to work with than many other foodstuffs.

3.1 Aroma isolation methods

3.1.1 Solvent extraction

By the use of solvents fruit juices can be extracted directly. The solvent containing the extracted volatiles is concentrated and a fraction injected on a gas chromatograph (GC). The method is simple and reproducibly but labour intensive. Low boiling compounds are lost in the solvent front in GC-analysis, but the technique is superior for isolation of high boiling compounds. Simultaneous distillation extraction (SDE) is often applied for lipid containing foods (43,44).

Nonpolar organic solvents are used for the extraction of volatiles from foods. The extraction efficiency of volatiles in the solvent phase depends on their affinity towards the solvent and hence partition between the food and solvent phases. Extraction solvents are usually selected on basis of selectivity and boiling point. Ether is extensively used because its extraction efficiency is high. Hydrocarbons are relatively non-selective but have low extraction efficiency, and pentane is a poor solvent for the extraction of polar substances like alcohols. Dichloromethane has higher extraction efficiency than pentane and ether, a disadvantage however of dichlormethane is its toxicity (43-45).

Direct solvent extraction with ether/pentane was used for extraction of black currant aroma compounds in paper II and VII, and solvent extraction was applied in (7,46-51) - in some cases combined with distillation.

3.1.2 Headspace sampling

During headspace sampling volatiles from the sample, placed in a closed vessel, are isolated from the atmosphere above it. An advantage is that there is no solvent peak in the chromatogram, except when solvent desorption is applied (52).

In static headspace equilibrium between the food and the headspace is reached, and only an aliquot of the atmosphere in the vessel is normally analyzed, resulting in low sensitivity. Only highly volatile compounds or medium volatiles in high concentrations are recovered. The procedure is easily automated and good reproducibility is achieved (44,52). Alternatively the entire volume of headspace is recovered by displacement and collected on traps, thereby increasing sensitivity as in (53) and paper II.

In dynamic headspace sampling and purge and trap (P&T) the sample is purged with an inert gas stream above or through the sample, respectively[3], continuously removing the headspace, shifting the sample/air equilibrium. The stripped volatile constituents are collected in a trap containing adsorbent material (see Section 3.1.3). It is a very sensitive method, offering good recoveries for the more volatile esters, but recovery of the higher boiling compounds is poorer relative to solvent extraction (43) depending on purge gas volume, as demonstrated in paper II. The coefficient of variation (CV) of this method is quite low for most compounds (43), which is confirmed in our studies except for terpenes (see Section 3.1.3). Dynamic headspace with mastication is closest to the situation during eating, resembling direct oral vapour release in the mouth (*54*).

Headspace sampling is used for isolation of black currant aroma by (47,55-63) and headspace sampling followed by thermal desorption is applied in paper I-VIII.

3.1.3 Adsorbent materials

The stripped volatile constituents of dynamic headspace are collected in traps containing an adsorbent material. In so doing, criteria of the adsorbent material are complete enrichment of the analytes, complete and fast desorption of the analytes, homogenous and inert surface. Of consideration is also breakthrough volume of volatiles on the adsorbent, i.e. the volume of gas that causes a compound to migrate through an adsorbent bed of one gram at a specific temperature. Desorption of the analytes trapped during headspace collection is done either thermally, as done in the present studies, or by the use of solvents. A comprehensive review dealing with commonly used adsorbent materials is given in (64,65). Properties of adsorbent materials have mainly been studied in relation to analysis of air (66-73).

[3] Never the less, the term dynamic headspace is used for bubbling the gas through the sample in paper I and III-VIII, only in paper II is the P&T terminology used.

A rough classification of adsorbent materials can be based on three categories (64):

- inorganic materials which are of minor practical importance because of the often high hydrophilicity of these materials
- carbon based adsorbents, sub-classified into activated carbon, carbon molecular sieves and graphitized carbon blacks such as Carbotrap and Carbopack
- porous organic polymers like Tenax

Despite the great variety of commercially available adsorbents a universal adsorbent does not exist, hence a possibility is to use a combination of sorbents (64). Tenax is perhaps the most widely used general purpose sorbent for dynamic headspace techniques (52). Compared to other adsorbent materials Tenax covers collection of compounds with a large range of molecular sizes (C7-C26), which is an important feature in the analysis of black currant aroma, constituted by many different volatiles (see Section 4.1). Tenax is characterised as a very hydrophobic material with a relatively high thermal stability. Tenax perform well in recovery, storage stability, and detection level (72) and it has low water retention (74). Tenax TA consist of poly(2,6-diphenyl-*p*-phenylene oxide) and Tenax GR consist of Tenax TA with 30% graphitized carbon blacks (64,65).

One drawback of Tenax is the tendency to form artefacts as a result of degradation of Tenax itself and breakdown of reactive compounds adsorbed on the adsorbent surface (64,74). Artefacts reported are acetone, benzene, toluene, benzaldehyde, octanal and nonanal, nonane and decane (69,74). Artefacts in the form of benzaldehyde, acetic acid, octanal, nonanal, decanal were also to a minor extend observed in the present studies (results not published).

Due to its low specific surface Tenax is not suitable for sampling of highly volatile organics and it is not suitable for very small alcohols (52,64,65). According to (75) Tenax GR has a higher breakthrough volume than Tenax TA for most volatile organics, but that was not confirmed in paper V. Breakthrough of black currant juice volatiles on Tenax GR and TA was reported in paper II, IV, and V (Table 2).

Table 2. Breakthrough of volatiles on Tenax TA and GR adsorbents reported in paper II, IV and V (not all published).

Compound	Tenax TA	Tenax GR
Ethanol	x	x
2-Methyl-1-propanol	x	x
2-Methyl-3-buten-2-ol	x	x
2,3-Butanedione	x	x
Methyl acetate	x	x
Ethyl acetate	x	x
Methyl 2-methylpropanoate		x
Dimethyl sulphide	x	x

For sampling of microbially produced volatiles Tenax GR had a larger catalytic effect than Tenax TA on breakdown of some compounds, and Tenax TA had the best overall properties of

eight adsorbents tested (73). On the contrary Tenax GR gave better yield than Tenax TA in indoor air monitoring of a mixture of 11 monoterpenes including linalool, limonene and other terpene hydrocarbons. The use of an ozone scrubber did not influence these results (72).

Irreproducibility of certain terpenic compounds in our laboratory in connection with analysis of black currant juice, including preliminary experiments of paper IV and separate analysis of berries (not published) with Tenax GR, occurred. For this reason a closer investigation of Tenax GR and Tenax TA properties in juice and model solutions (paper V) was commenced. It was demonstrated that the black currant juice terpene alcohols linalool, α-terpineol and 4-terpineol are subject to degradation on Tenax GR, resulting in the formation of terpene hydrocarbon breakdown products. **Linalool** was most affected and converted mainly to myrcene and limonene, **α-terpineol** was converted mainly to limonene, terpinolene, *p*-cymenyl and *p*-cymene, and **4-terpineol** was converted to *p*-cymene. The terpene hydrocarbons **limonene** and ***p*-cymene** were not degraded where as **α-terpinene** on the other hand was oxidised to *p*-cymene on both TA and GR, indicating that an acid catalysed rearrangement take place on the surface of the sorbent (Figure 5). Except α-terpinene no considerable breakdown of compounds was observed with Tenax TA. None of the other compounds identified in black currant juice showed irreproducibility upon sampling on either Tenax TA or Tenax GR. Decomposition of terpene alcohols was larger on old than on new Tenax GR, but only small differences were observed between old and new Tenax TA. However, higher degradation of monoterpenes on old than on new Tenax TA has been reported (67).

From paper V it was concluded that Tenax TA is more suitable than Tenax GR for determination of terpenes in black currant juice and in the following experiments Tenax GR was replaced by Tenax TA. Subsequently however in paper IV and VII, Tenax TA resulted in larger relative standard deviations of terpene hydrocarbons than of the other compounds identified (partly published).

Several factors can be involved in the observed terpene degradations and these are considered in the following:

- High relative humidity in the headspace of food products collected on Tenax caused losses of 4-terpinol, linalool and the other terpineols measured, whereas apolar compounds such as terpene hydrocarbons were not affected. This was explained by interactions between volatile compounds, water and the adsorbent trap during the P&T step due to break through of compounds on the trap (74). However, this does not apply to our studies, as break through of terpineols does not occur (Table 2, p. 14).
- Oxidative decomposition on Tenax of terpenes in air correlate well with the compounds' reaction rate with ozone which is correlated to their degree of unsaturation (66,68).
- Sampling of terpenes from air on acid treated Tenax leads to decrease of α-pinene and β-pinene and formation of limonene, camphene, terpinolene and *p*-cymene indicating acid catalysed rearrangements (68) (Figure 5). Degradation of monoterpene hydrocarbons on Tenax TA resulting in formation of terpenes and aromatic compounds suggested rearrangement and dehydrogenation reactions. Desorption temperature, storage duration of traps or concentration of aroma does not influence the artefact formation (67).

- Graphitized carbon blacks, made out of soot, are highly hydrophobic and pure adsorbents, but there are indications of active sites on the surface (64,76), and the presence of graphite in the adsorbent of Tenax GR was suggested to give rise to irreproducible results and losses of terpenes in the determination of forest air (70).

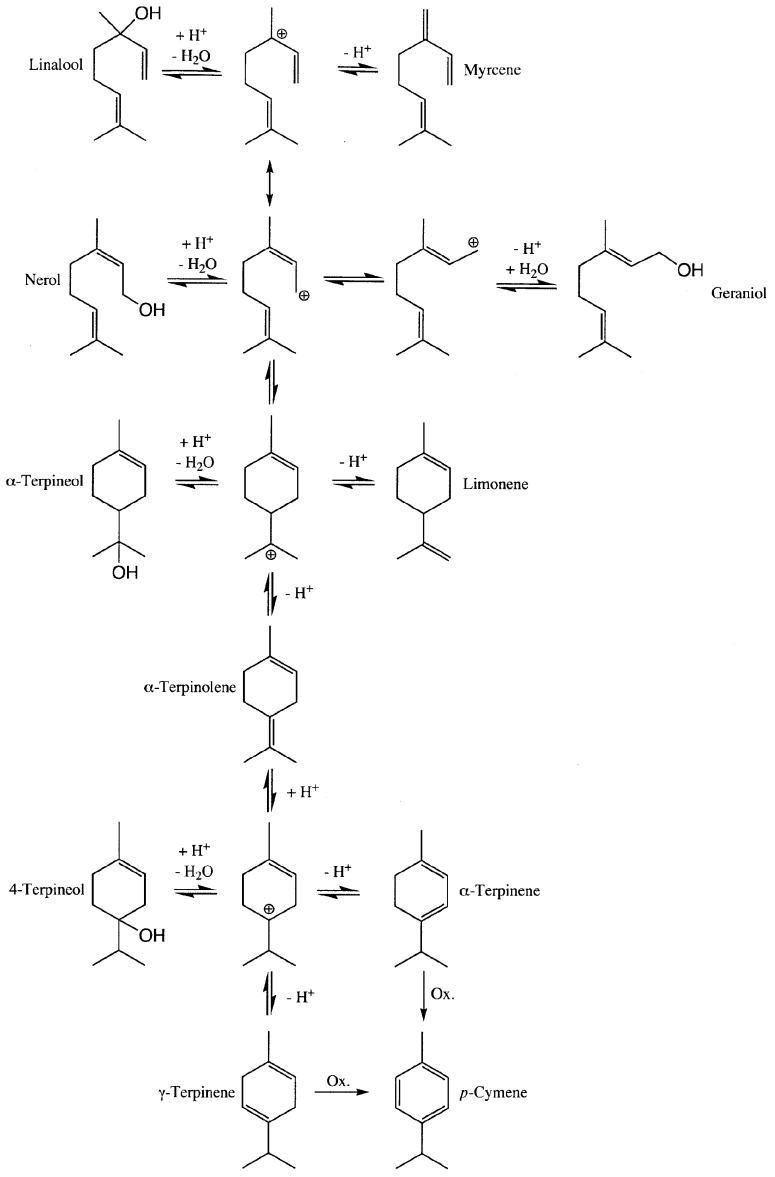

Figure 5. Acid catalysed rearrangement of terpenes (77).

3.1.4 Detection of volatiles

GC coupled with mass spectrometry (MS) is the predominant method used to separate and identify volatiles from most foods, including black currant. High performance liquid chromatography (HPLC) is suited for separation of thermally unstable compounds and can be used for less volatile constituents or the non-volatile glycosidically bound aroma compounds, as well as for taste compounds (78,79). HPLC has been used to separate volatile mixtures of black currant buds (80,81).

Aiming at identifying unknown important aroma compounds in paper II, a nitrogen specific detector (GC-PND) following freon extraction of black currant volatiles was tested preliminary by another laboratory (not published). The resulting chromatogram contained 11 peaks of varying size, contributing the tentative identification of 2-methoxy-3-isopropylpyrazine and 3-methoxy-2-isobutylpyrazine of importance according to GC-olfactometry (GC-O) (paper II).

3.2 GC-olfactometry

Many of the volatile components in a typical gas chromatogram are not aroma active. In GC-O the human nose is used as detector for evaluating the effluent of the GC-column to select compounds that contribute to the aroma of a food. Several techniques have been developed to objectify GC-O data and to estimate the sensory contribution of single aroma components (Table 3) (82,83). In Osme the perceived odour intensity of a compound is rated. Combined hedonic and response measurement (CharmAnalysis) and aroma extract dilution analysis (AEDA) are GC-O methods often used. These methods are based on one or a few assessors sniffing stepwise dilutions of a solvent extract until no odours can be detected. The nasal impact frequency (NIF) method uses only one dilution level, but GC-O is repeated by a number of panellists, i.e. data treatment is based on detection frequency rather than successive dilutions (84,85). For determination of important, or impact, aroma compounds in black currant juice (paper II) the NIF method was chosen as large variation among subjects require more than one or a few assessors for reliable GC-O results (11-14), and the method correlate well with sensory odour intensities (13).

Table 3. Summary of GC-O techniques for determination of aroma active compounds in foods.

Technique		Principle
Time-intensity	Osme (86)	Rating of the odour intensities by e.g. four judges at one concentration level.
Dilution methods	AEDA (87) and CharmAnalysis (88)	One or a few judges sniffing series of dilutions of a solvent extract.
	Static headspace (89)	One or a few judges sniffing series of: - various injected headspace volumes.
	Dynamic headspace sampling (90)	- decreasing purge gas volumes.
Detection frequency	NIF (85)	Several panellists sniffing one dilution level of a sample.

Various GC-O methods, following different isolation methods of black currant aroma compounds, have been reported. Paper II and (57) employed the NIF-method with nine judges after P&T with thermal and solvent desorption, respectively. In (42) nine judges sniffed SPME isolates also according to the NIF-method. In paper I, three judges sniffed samples of P&T with thermal desorption and evaluated them in a manner similar to the Osme method. In (61) two judges classified odours of a dynamic headspace solvent desorption extract as weak or strong, and in (48) 15 judges sniffed a distilled extract.

Limitations of GC-O are contrast effects depending on elution order of compounds, human fatigue, sensory saturation and adaption. In addition, sensory perception of a food is arrived at by simultaneously integration of all the taste and aroma compounds in a food, but GC-O odours are evaluated out of context. Alternatively, odours can be evaluated by aroma-recombination studies; and correlation of GC-O data to sensory methodologies is useful (83).

3.2.1 Influence of aroma isolation method on GC-O results

The success of flavour characterisation of foods by GC-O depends largely on the aroma isolation method employed (83). In paper II the influence of isolation method on the determination of important aroma compounds in black currant juice was investigated by the NIF technique. The applied isolation methods were solvent extraction, static headspace and P&T using fifteen (P&T 15) and sixty minutes (P&T 60) purge time. Most odours were observed by P&T 60 followed by solvent extraction, P&T 15 and static headspace (paper II, table 1). P&T 60 and solvent extraction lead to relatively more odours with high retention indices (RI) than static headspace and P&T 15. Sixteen odours were observed by P&T 60 only. Static headspace sampling and P&T 15 differed from P&T 60 particularly in that a lower number of esters and aldehydes, and no phenolics, were observed. Six odours corresponding to less volatile compounds were only observed by solvent extraction. The relative number of esters and terpenes observed by solvent extraction was lower than for the headspace methods. The relative importance of compounds within each method according to their surface of nasal impact frequency (SNIF) values was evaluated (paper II, table 2). Ethyl butanoate was the only compound ranked in top five by all methods, and 2,3-butanedione was represented by the three headspace methods. Static headspace and P&T 15 ranked more esters, and P&T 60 and solvent extraction ranked less volatile compounds. Relative to the headspace methods, solvent extraction was dominated by fewer compounds representing fruity odours.

Comparable results have been reported for the GC-O analysis of cooked tail meat of freshwater crayfish. Decreasing headspace of purge and trap and static headspace gave similar results for the most volatile compounds, whereas AEDA of vacuum steam distillation-solvent extraction was characterized by identification of mainly intermediate- and low- volatility compounds (90). In the analysis of cooked tail meat of American lobster several highly volatile compounds found by purge and trap and static headspace were not detected in solvent extractions and the reverse was true for the higher boiling compounds (91). In a study concerning tea powder, the majority of the compounds identified by static headspace were also identified by AEDA, but by AEDA several additional compounds were identified (89). Comparisons between isolation methods

however are difficult to make as different sample amounts are used, influencing the detection limit.

4. Aroma in black currant

Aroma of intact fruit is mostly produced as part of the normal metabolism of the plant, and production of fruit volatiles occurs mainly during a short ripening period (92). The aroma profile of black currant shares similarities with other berry fruits, but black currant are more abundant in terpenes (93). In the 1960ies some of the earliest studies of black currant aroma were published by von Sydow and co-workers (55,63,94).

4.1 Aroma compounds identified in black currant berry and juice

Several papers have dealt with the issue of identifying the aroma constituents of black currant berry and juice and a total of 240 compounds have been reported (Table 4). Terpene hydrocarbons and oxygenated terpenes in the form of, mainly mono -, and sesquiterpenes constitute the largest group of compounds, followed by esters and alcohols. There is a large variation in the compounds reported within berry and juice, respectively. These differences can be explained by different aroma isolation methods involved (Section 3.1), the parameters applied in juice processing (Section 2.2) and to a smaller degree, variety and maturity of the berries (Section 4.1.1). For the same reasons some caution must be taken interpreting differences between berry and juice in Table 4, as it is not the concrete berries evaluated that are trailed in the processed juice.

The highest number of terpene hydrocarbons is identified in the berries - owing to their hydrophobic nature they are lost in the pressing cake during juice processing (Section 5.1). Of the other compound groups the highest number are identified in the juice (even though the concentration of some compounds decreases in juice processing, see Section 5.1). Reasons for this can be that aroma components are released from the cells in juice processing, or can easily escape from the sample matrix during aroma isolation, as well as compounds are formed in processing (Section 5.1).

Aroma compounds in berry (47,50,58) and in juice (paper IV, table 1) and (61) is quantitatively determined to be in the one ppb to a few ppm range.

Table 4. Aroma compounds identified in black currant berry and juice.

Compound	Identified by GC-MS		Identified by GC-O	
	Berry	Juice	Berry	Juice
Alcohols				
Ethanol	(46,55,57)	(51)[a]		
1-Propanol	(55)	(51)		
2-Methyl-1-propanol	(46,55,57,58)	pIV[b], pVIII (7,51)		
1-Butanol	(46,55,57)	PI, pVIII (7,48,51)		
2-Butanol	(55)	pVIII		
2-Methyl-1-butanol	(57)	pI, pIV		pII[d]
3-Methyl-1-butanol	(46,55)	pI, pIV, pVIII (51)		pII[d]
2-Methyl-3-buten-2-ol	(55)	pIV (7,51,61,62)	(57)	

Table 4 continued…	Berry GC-MS	Juice GC-MS	Berry GC-O	Juice GC-O
3-Methyl-2-buten-1-ol	(55)	(7)		
2-Ethylbutanol		(51)		
1-Pentanol	(55)	pI, pVIII (7,48,51)		
2-Pentanol	(55)	pVIII (7,51)		
3-Pentanol		pVIII		
1-Penten-3-ol	(55)	pVIII (7)		
Methyl-2-pentanol				
1-Hexanol	(46,47,55,57)	pI, pIV, pVIII (7,48,51)		
2-Hexanol		(51)		
3-Hexanol		(48)		
trans-2-Hexen-1-ol	(46,47)	pI, pIV, pVIII (48,51)		
Cis-3-Hexen-1-ol	(94)	pI, pIV, pVIII (48)		pI, pII[d]
2-Ethyl-1-hexanol	(46)	pI		
Cyclohexanol		(48)		
1-Heptanol		pI, pVIII (7,48)		
1-Octanol	(57)	pI, pIV, pVIII (57)	(57)[c]	
4-Octanol		(51)		
1-Octen-2-ol		(48)		
1-Octen-3-ol	(57,94)	pVIII (48)		(48)[f]
1-Nonanol		pI (48)		
Aldehydes				
Ethanal	(55,57)	(62)		
Propanal		(62)		
2-Methylpropanal		(62)		
2-Methylbutanal	(47,55,57)	(62)		
3-Methylbutanal	(47)			
trans-2-Methyl 2-butenal		pI, pIV		
2-Methyl-3-butenal		(7)		
3-Methyl-2-butenal	(47)			
Pentanal	(47,55)	(62)		
Hexanal	(47,55,57,58)	(48,61)	(42)	
(*E*)-2-Hexenal	(46)	(51,61,62)		(61)
Heptanal		(48,61)		
(*E*)-2-Heptenal		(61)		
Octanal		pIV (48,61)		pII
(*E*)-2-Octenal	(57)	(61)		
Nonanal	(55,57)	pIV (61,62)		pII (61)
(*E*)-2-Nonenal			(57)	pII
Decanal	(55,57)	(48,56)		pII[d]
Ketones				
2-Propanone	(57)			
2-Butanone	(55)			
2,3-Butanedione	(55)	pIV		(48) pII
3-Methylbutan-2-one		(51)		
3-Hydroxybutan–2-one		(7)		
2-Pentanone		pIV (51)		
(*E*)-3-Penten-2-one		pIV (7)		

Table 4 continued…	Berry GC-MS	Juice GC-MS	Berry GC-O	Juice GC-O
2-Methyl-3-pentanone		(48)		
2-and 3-Methylcyclopentanone		(48)		
2-Hexanone	(46)	(48)		
3-Hexanone	(47)	(48)		
2-Heptanone		(48)		
6-Methyl-5-hepten-2-one		pIV		pII
2-Methyl-2-hepten-4-one		(48)		
3-Octanone		pI (48)		
1-Octen-3-one		(61)	(42,57)	pII (61)
1,4-Dimetyl-3-cyclohexenyl-methylketone		pI		pII[d]
Acetophenone		(48)		
Methyl acetophenone		(48)		
Esters				
Methyl acetate	(46,55,57)	pIV (51)		pII
Ethyl acetate	(46,55,57,58)	(51,61,62)		
2-propyl acetat		(51)		
2-Methylpropyl acetate		pIV (48,51)		pII[d]
Butyl acetate	(46,55,57)	pI, pIV (48,51)		
2-Methylbutyl acetate		pIV		pII[d]
3-Methylbutyl acetate	(46)	pI (48,51)		pI, pII[d]
Pentyl acetate	(57)	(51)		
Hexyl acetate		pI, pIV (48,61)		pII
2-Hexenyl acetate	(57)	pI, (48)		
Cis-3-Hexenyl acetate	(57)	(48)		
Octyl acetate	(57)			
Ethyl propanoate		pIV		pII
Methyl 2-methylpropanoate		pIV		pII
Methyl butanoate	(57,58)	pI, pIV (7,51,56,57,61)	(42,57)	pI, pII (61)
Methyl butenoate		(61)		
Methyl 2-butenoate		pI (48)		pII
Methyl 2-methylbutanoate		pIV		pII[d]
Ethyl butanoate	(46,55,57,58)	pI, pIV (7,57,61)	(42,57)	pI, pII (48,61)
Ethyl 2-butenoate		pI		
Ethyl 3-methyl butanoate		pIV	(42)	pII
Butyl butanoate	(57)			
Methyl hexanoate	(46,57,58)	pI, pIV (7,51,61)	(42)	pI
Ethyl hexanoate	(46,57,58)	pI, pIV (56,57)	(57)	pI, pII (61)
Methyl-2-hexenoate		pI		
Methyl octanoate	(57)	pIV (48,56,61)		pII
Ethyl octanoate	(57,58)	(48,56,61)		(61)
3-Methylbutyl octanoate		(56)		
Methyl decanoate	(57)	(56,61)		(61)
Ethyl decanoate	(57)	(56,61)		
2-Methylbutyl decanoate		(56)		
3-Methylbutyl decanoate		(56)		
Methyl dodecanoate	(57)	(56)		

Table 4 continued…	Berry GC-MS	Juice GC-MS	Berry GC-O	Juice GC-O
Ethyl dodecanoate		(56)		
Ethyl tetradecanoate		(56)		
Methyl 2-hydroxybutanoate		(7)		pII a
Methyl 3-hydroxybutanoate		(7)		
Ethyl 2-hydroxybutanoate		(7,48)		
Methyl 2-hydroxyhexanoate		(7)		
Methyl 3-hydroxyhexanoate		(7)		
Methyl acetoacetate		(48)		
Terpene hydrocarbons				
α-Pinene	(41,50,55,57,58,94)	pVII (51,57,61)	(42,57)	
β-Pinene	(41,50,55,57,58)	(61)	(42)	
α-Terpinene	(50,55,57,58,94)	pI, pIV, pVII (51,56,61)		
γ-Terpinene	(41,46,50,55,57,58,94)	pI, pIV, pVII (51)	(42)	
Limonene	(41,50,55,57,58,94)	pIV pVII, (51,61)	(42)	(61)
Terpinolene	(41,50,55,57,58,94)	pI, pIV, pVII (62)	(42)	
Phellandrene	(58)	pIV		
α-Phellandrene	(41,46,50,57,94)	pI (51,61)	(42)	
β-Phellandrene	(55,57,94)	(51,61)		(61)
Myrcene	(41,46,50,55,57,58,94)	(51,61,62)	(42)	
Sabinene	(41,50,57,58)		(42)	
Sabinene hydrate	(50)			
Thujene	(50,55,58)			
α-Fenchene	(55,57)			
Ocimene	(50,55,57,58,94)	(51,61)	(42)	
Neo-allocimene	(57)	(57)	(57)	
3-Carene	(41,50,55,57,58,94)	pIV (61,62)	(42)	
(+)-4-Carene	(47)			
p-Cymene	(41,55,57,58,94)	pI, pIV pVII (51,61,62)		
m-Cymene	(94)	(56)		
p-Cymenyl		pI		
Dehydro *p*-cymene	(57)			
Caryophyllene	(41,55,57,94)			
α-Caryophyllene		(61)		
β-Caryophyllene	(50,57,58)	(61)		
Humulene	(41,50,55,57,94)			
γ-Elemene	(50,55)			
δ-Cadiene	(50,55,57)			
Camphene	(41,50,94)	(51)		
Alloaromadrene	(50)			
Germacrene	(50,57)			
α-Copaene	(57)			
β-Cubebene	(57)			
Muurolene	(57)			
Terpenoids				
Linalool	(41,50,57)	pI, pIV, pVII (7,48,51,56)		pI (48)
4-Terpineol	(41,46,50,55,57,58,94)	pI, pIV, pVII (48,51,56,57,61)	(57)	pI, pII (48)
α-Terpineol	(41,46,50,55,94)	pI, pIV, pVII (7,48,56,61,95)		pII (48)

Table 4 continued…	Berry GC-MS	Juice GC-MS	Berry GC-O	Juice GC-O
β-Terpineol	(50)			
γ-Terpineol	(50)			
Menthol		pVII		
Neomenthol		pVII		
Geraniol	(41,50)	(48,51)		(48)
Citronellol	(41,55,57,94)	pVII (48,51)		(48)
Limonen-4-ol		pVII, pI (48)		(48)
Limonene-1,2-diol		(7)		
Nerol	(50)			
Anethol	(57)			
Piperitol	(50)			
Pinocarveol		(56)		
m-4,6-Menthadiene-8-ol	(47)	(7)		
p-Cymen-8-ol	(41,55,57,94)	pVII, pI (7,48,51)		
α-Cyclocitral		pI		
β-Cyclocitral		pI, pIV, pVII		
Safranal		pI		
Phellandral		pI, pVII		pII
p-Mentha-9-al	(47)	pI		
Geranial	(47)			
2-Caren-10-al				
Cumin aldehyde		pI, pIV, pVII		
Menthone		pVII		
Isomenthone		pVII		
Camphor		pIV (48)		
Piperitone	(47)	(48)		
Carvone		pI (48)		
Eucarvone		(56)		
Pinocarvone		pI		
1,4-Cineole		pI, pVII		pII
1,8-Cineole	(41,46,55,57,58)	pI, pIV, pVII (7,48,51,57)	(57)	pI (48)
Exo-hydroxycineol		pVII		
Rose oxid	(55,57)	pI, pIV, pVII (48,57)	(57)	pII
cis-Linalool oxide		pIV pVII (7,48)		
Bornyl acetate	(50,57)	pI, pVII (48,56,61)		
Citronellol acetate	(47,57,94)	pI (48,56)		
Terpinyl acetate	(47)	(48,61)		
Fenchyl acetate	(50)			
Linalool acetate	(50,57)	(48)		
Geranyl acetate		(48)		
α-Nerolidol	(50)			
(+)-Spathulenol				(48)
β-damascenone		pI, pIV, pVII (48,56,57)	(57)	PI, pII (48)
β-ionone		(56)		pII
Epoxy-5-6-8-ionone		(48)		
Vitispirane		pI		
Caryophyllene oxide	(50,57)			

Table 4 continued…	Berry GC-MS	Juice GC-MS	Berry GC-O	Juice GC-O
Phenols				
Phenol		pI (48)		
4-Methylphenol		pIV		pII
4-Vinylphenol				(48)
Eugenol		pIV, pVIII (7,48,61)	(57)	pII
Carvacrol		pIV		
Thymol		pI		pI
p-Cresol		(48)		
Vanillin		(48)		
2,4-bis-tert-Butylphenol		pVIII		
4-Vinyl-2-methoxyphenol				pII
2,6-bis(1,1-dimethylethyl)-4-methyl phenol		(56)		
Furans				
2-Acetylfuran		pI, pIV		
Methyl isopropylfuran		(48)		
2-Pentylfuran	(57)			
Furfural		pI, pIV (7)		
Furfuryl alcohol		pI (48)		
Methyl-2-furoate		pI, pIV		
Benzene derivatives				
Toluene			(42)	
Butyl benzene	(47)			
1-Methyl-4-isopropenyl-benzene	(46,47)	(51,62)		
Benzylalcohol		pIV, pVIII (7,48)		pII
Phenylethanol		(7,48)		pII
2-phenylethanol		(56)		pII
Benzaldehyde	(47,94)	pI, pIV, pVIII (7,50,51)		
p-Methoxy-benzaldehyde		(48)		
4-Methylacetophenone				(48)
Benzylacetate		(48)		
Methyl benzoate	(46,47,94)	(48,51,61)		
Ethyl benzoate	(47,57,94)	pI, pIV (50,56,61)		
Methyl salicylate	(94)	pI, pIV, pVIII (48,51,56)		
Ethyl salicylate		pI		
Sulphur				
Dimethyl sulfide		pIV (62)		pII
Methional				pII
4-Methoxy-2-methyl-2-mercaptobutane			(57)	
2-Methyl-3-furanthiol				pII
Benzothiazol		(48)		
Pyrazines				
3-Methoxy-2-isobutylpyrazine				pII
2-Methoxy-3-isopropylpyrazine		pIV	(57)	pII
Acids				
Acetic acid		(7,56)		pII
Butanoic acid		(56)		pII

Table 4 continued…	Berry GC-MS	Juice GC-MS	Berry GC-O	Juice GC-O
2- and 3-Methyl butyric acid				pII
Hexanoic acid		(56)		
Octanoic acid		(7,56)		
Lactones				
Methyl-2-buten-2-olide		(48)		
γ-Butyrolactone		(48)		
ε-Caprolactone		(7,48)		
γ-Caprolactone		(48)		
γ-Nonalactone		(48)		
δ-Decalactone		(48)		

[a] 100 fold aroma distillate. [b] p=paper. [c] A homogenised fluid pressure filtered extract of berry, not subject to further juice processing treatments. [d] Uncertainty of which compound of more co-eluting compounds was responsible for the odour. [e] Nectar. [f] GC-O performed on an alcoholic preparation sample.

Distinct compartmentation in the distribution of mono - and sequiterpenes in black currant berry has been reported (50). The majority of the terpene compounds were found in the epidermis (particular the mono - and sequiterpene alkenes), and a relative high level of monoterpene alcohols were found in the pericarp, and to a lesser extend the seeds, indicating that the epidermis is the major site of terpene synthesis.

The essential oil obtained by extraction of black currant buds is used as flavourings and fragrance material. Terpenes constitute the vast majority of the compounds identified in buds, of which the greater part has also been identified in berry (96-98).

4.1.1 Factors influencing the aroma in black currant berry

Influence of maturity on aroma compounds in black currant berry was investigated by von Sydow and associates (63). They found that the monoterpene fraction and the total amount of essential oil in the berries increased slowly during ripening. The concentration of 3-carene, terpinolene, citronellyl acetate and caryophyllene increased, where as 4-terpineol did not change. Of the more volatile compounds the majority changes very little, except ethanol and 2-methyl-3-buten-2-ol, which increased considerably during the later part of the ripening process (63). Marriot (50) investigated distribution of terpenes during ripening and found that the changes were quantitative rather than qualitative. Contrary to the result of Sydow (63) an overall decrease in total terpenes as ripening progressed was observed, with a relative increase in monoterpene alcohols in the latter stages. However, it is generally understood that the flavour development period occurs in the ripening stage, where minute quantities of carbohydrates, lipids, and proteins and amino acids are converted to volatile flavours (21,92). Soil cultivation and nitrogen supply has no significant effect on production of volatile compounds in black currant (58), but seasonal and geographical effect on black currant concentrates has been reported (99).

Differences between varieties of black currant were found to be quantitative rather than qualitative, with large quantitative variation in aliphatic esters and monoterpenes (58). Also (63) reported differences in the concentration between all examined varieties for some aroma compounds. A study of the differences in terpene composition of ten black currant cultivars

revealed differences in proportions rather than in qualitative composition. 1,8-cineole and α-terpineol exhibited great variability, 4-terpineol varied less and differences between the other monoterpene alcohols were negligeble. Monoterpene hydrocarbons were generally detected in greater proportions than the alcohols. All monoterpene hydrocarbons considered exhibited similar enantiomeric composition in all varieties, which may be used as a "fingerprint" for the presence of black currant i.e. authenticity control. In contrast some of the monoterpene alcohols displayed a large variation and 4-terpineol was suggested used as a marker to distinguish between cultivars (41).

Sensory differences between cultivars of black currant was studied, and significant genotypic variation of black currant juice was found in all the main sensory characters appearance, flavour, aroma, mouth-feel and aftertaste, with by far the most important variation detectable in the flavour component (100).

4.2 Important aroma compounds in black currant berry and juice

A range of compounds is of importance to black currant berry and juice aroma, but these constitute only a fraction of the total number of compounds identified (Table 4). Variations in compounds reported are in addition to the reasons mentioned in Section 4.1 due to different GC-O methods applied (Section 3.2). Regarding the number of important odours representing aroma compound groups in berry versus juice the same apply as for identified compounds: only terpene hydrocarbons are more numerous in berry and of the other groups more important compounds are found in the juice. Several of the odours in paper II and (42,48,57), mainly compounds of medium to low volatility, could not be identified, meaning that they are isolated in concentrations below the GC-MS detection limit but above the sensory threshold of GC-O.

Esters and (oxygenated) terpenes comprise the largest compound groups of importance to black currant odour. Relative to the total number of compounds identified within each group, a large fraction of the esters and sulphides are important. These compounds have low odour thresholds values (101). In contrast, alcohols and furans are not significant contributors owing to their high threshold values.

Compounds found important for either black currant berry or juice by at least two of the references in Table 4 is compiled in Table 5. All compounds, except α-pinene, important for berry aroma are also important for juice aroma, but only some of the compounds important for juice are also important for berry. However, the fewer studies on berry than juice has to be taken into account. The esters are responsible for similar odour characteristics related to fruity notes and the terpenes for various aromatic odours.

By GC-O a "catty note" of importance to black currant odour was first reported by Latrasse et al. (1982) (48), but no compound responsible for the odour could be detected. The compound was later identified in black currant buds as 4-methoxy-2-methyl-2-mercaptobutane, with a perception threshold in water of only 10^{-6} ppm (102). The compound was tentatively identified in black currant berry by (57) (Table 4), and in GC-O of black currant berry a catty note has also been observed but not identified by (42). In paper II, table 1, the odour description "cat urine"

was given at RI 1191. No traces of the compound in the GC-chromatogram could be detected, however the RI is close to that of 4-methoxy-2-methyl-2-mercaptobutane (103).

Table 5. Compilation of compounds determined by GC-O as most important for black currant aroma.

Compound[a]	Odour characteristic[b]	Berry	Juice
2,3-Butanedione	Butter, caramel	-[c]	pII [d] (48)
1-Octen-3-one	Mushroom	(42,57)	pII (61)
Nonanal	Library, flower, citrus	-	pII (61)
2-Methylbutyl acetate	Banana, chewing gum	-	pI, pII
Methyl butanoate	Fruit, sweet, chewing gum	(42,57)	pI, pII (61)
Ethyl butanoate	Fruit, sweet, chewing gum, strawberry	(42,57)	pI, pII (48,61)
Ethyl hexanoate	Fruit, orange, sweets	(57)	pI, pII (61)
α-Pinene	Conifer	(42,57)	-
1,8-Cineole	Menthol, balsamic	(57)	pI, pII (48)
Linalool	Floral	-	pI, pII (48)
4-terpineol	Moldy, terpenic	(57)	pI, pII (48)
α-Terpineol	Terpenic, green	-	pII (48)
β-Damascenone	Boiled fruit, black currant juice/ribena	(57)	pI, pII (48)

[a] Compounds important for black currant aroma referred by at least two references for either berry or juice in Table 4. [b] Most frequent odour characteristics mentioned in the references. [c] Not observed by GC-O. [d] p = paper.

Commercially available products of black currant products sometimes contain Buchu oil, with a catty odour caused by a different sulphur compound than 4-methoxy-2-methyl-2-mercaptobutane, without any indication on the label. Analysis of the product by GC-O or by a sulphur selective detector can prove what compound is truly responsible for the odour. The occurrence of other compounds characteristic of Buchu oil, not present in black currant are also suggested as indication of adulteration (104).

Piggott et al. 1993 (105) reported that variation in flavour intensity of black currant drinks measured by sensory evaluation appeared to be due to simultaneous variation in a large number of aroma compounds rather than to changes in a small number of impact compounds.

4.3 Glycosidically bound aroma compounds

Many fruits contain non-volatile precursors of aroma compounds in the form of glycosidically bound compounds, with a biosynthetic function in the fruit (106,107). The glycosidically bound aroma compounds can be released from the precursors by heat facilitated acid hydrolysis, and/or enzyme hydrolysis (108) (Figure 6).

In paper VII and VIII the amount and identity of released bound volatile compounds of black currant juice from the corresponding glycosides was studied enzymatically using β-glycosidase in two different manners. β-glycosidase was added either directly to the juice, or to an extract of

glycosidic compounds that had been isolated from the juice by chromatography using Amberlite XAD-2. These two methods gave the same patterns of released volatile aglyconic compounds.

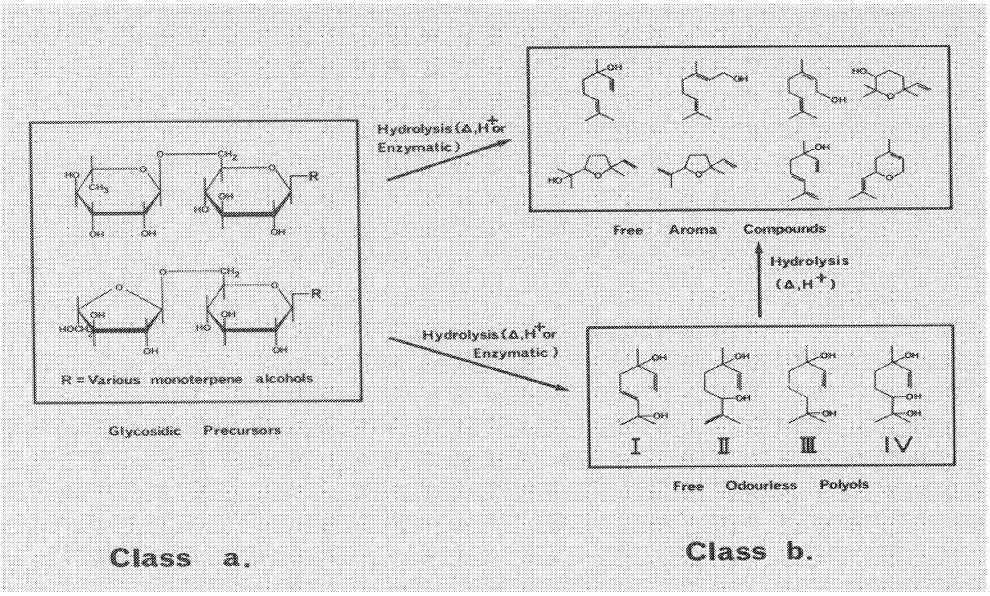

Figure 6. Monoterpene glycosides. Class a) Non-volatile glycosidically bound compounds; Class b) Free aroma compounds (108).

Fifteen aliphatic alcohols and five oxygenated aromatic compounds (paper VIII, table 1) as well as seven oxygenated terpenes (paper VII, table 3) were identified as glycosidically bound in the black currant juice. All compounds, except *p*-menthene-9-al and 3-caren-10-al which are not reported in black currant by any author (Table 4), were also present as free in the juice. For the majority of compounds the glycosidically bound fraction was larger than the free fraction, but a large variation in the amount and proportion of the glycosidically bound volatiles in the black currant juice was seen, and no obvious pattern was recognized. The glycosidically bound compound measured in the highest concentration was benzyl alcohol followed by eugenol, 2-pentanol, and 1-hexanol. Most of the identified glycosidically bound compounds have been reported as aglycones in other fruits (106).

Some of the glycosidically bound alcohol containing compounds identified in paper VIII is of importance to the odour of black currant juice, i.e. 3-methyl-1-butanol, *cis*-3-hexen-1-ol, 1-octen-3-ol, 1-octanol, benzyl alcohol, and eugenol (see Table 4). Glycosidic release might lead to the concentration of additional compounds exceeding their odour threshold values. Hence, release from a pool of glycosidically bound alcohols by enzymatic hydrolysis might lead to changes in the sensory impression of black currant juice. Of the released terpenes only geraniol are important for the aroma of black currant juice (48) (Table 4).

Evidence of glycosidically bound aroma compounds in black currant berry has previously been reported by Marriott (49) who found monoterpene alcohols and their corresponding alkenes in a ratio of 5:1. In that study the predominant aglyconic terpene alcohols were 4-terpineol and α-terpineol. Evidence of phenyl ethanol, benzyl alcohol and *p*-cymene-8-ol was also indicated (not published). Of these compounds only benzyl alcohol was found to be glycosidically bound in paper VII and VIII. Nevertheless, the terpene alcohols linalool, 4-terpineol and α-terpineol identified in black currant juice are glycosidically bound in many other fruits (106), hence these and other bound compounds might have been released in earlier steps of the juice processing. More over, Marriott (49) observed that the level of glycosides during ripening started to increase at the stage of complete sugar accumulation and then continued to rise during over-ripening.

5. Aroma changes during processing of black currant juice

Processing involve major structural changes in fruits (21), and when the tissue of fruit is disrupted in processing many volatiles are produced by enzyme and oxidation reactions. Thermal treatment of the fruit produces a whole new group of volatile compounds resulting from breakdown of carbohydrate, protein and lipid (109). Thus, the initial aroma characteristic of the intact fresh fruit is subject to alteration during juice processing.

The processing of black currant berry to juice involves several processing step, and thermal treatment is a major part involved in juice processing and conventional juice concentration (Chapter 2). Each step influences to a certain extend the aroma composition of the fruit depending on the parameters applied.

5.1 Aroma changes in juice processing

5.1.1 Pressing, enzyme treatment and pasteurisation

The influences of processing steps on various different aroma compounds are summarised in Table 6. Enzyme treatment and pressing mainly leads to loss of aroma compounds, whereas heat treatment causes formation of compounds.

Table 6. Summary of aroma changes in black currant juice processing.

Processing step	Compounds decreasing	Compounds increasing
Enzyme treatment	Esters (61) Terpenes (57,61)	
Pressing	Ethyl hexanoate, 1,8-cineole, β-damascenone (57) Monoterpene hydrocarbons (57,62) Aldehydes (62)	Aldehydes, ethyl acetate (62)
Heating or pateurisation of berry or mash	Octanol, esters (57) α-Thujene, α-pinene, 3-carene, limonene, β-phellandrene, γ-terpinene, myrcene, terpinolene (47,62)	Octanol, β-damascenone 4-terpineol (57) Dimethyl sulphide, carbon disulfide, 2-methyl-3-buten-2-ol, aldehydes, α-terpinene, α-phellandrene, linalool oxide, carveol, furan derivatives, benzene derivatives (47,62)

Pectinase enzyme treatment of blended black currant berry caused a decrease of several esters (61) (Table 6), however this observation might also have been contributed to by endogenous enzymes as no control was included.

It was reported that pressing of black currant mash leads to a decrease in the concentration of most volatiles, in particular monoterpene hydrocarbons (62) (Table 6). This was explained by

seeds and peels remaining in the press residue, containing lipid material, in which the hydrophobic terpenes are more soluble. In this experiment there was no report of a control either.

Mikkelsen and Poll (57) measured the changes in concentration of ten impact aroma compounds in pilot scale black currant juice processing (Table 6). Apart from β-damascenone increasing in concentration, an overall decrease of aroma compounds occurred through juice processing. The heating and pasteurization steps were the most influential, although evaporation from the semi open system employed might partly explain some of the observed losses. In the pressing cake more hydrophobic compounds were recovered, and in the juice more hydrophyllic were recovered. 4-terpineol and octanol increased during pressing as revealed by their excessive presence in the pressing cake. The compounds were unaffected by crushing and clarification, and by filtration only β-damascenone decreased.

In a series of experiments von Sydow and co-workers investigated the influence of heat on aroma and sensory quality of black currant mash treated with pectolytic enzymes (47,59,60,62). They observed formation of aromatics in addition to large rearrangements in the terpene complex (Table 6) but no breakdown of the terpene skeleton. The changes in the temperatures were larger at higher temperatures than at lower (62,110). GC-O evaluation characterized strongly heated samples as sickly, sulphurous, burnt and aldehyde (59). This was confirmed by sensory evaluation where similar odour qualities contributing undesirable qualities to a fresh fruit like cooked, pungent, sickly, musty, mould, and smoky aromas, increased with heating; while fruity, floral, and green decreased. The unpleasant odour qualities were positively correlated with the compounds increasing with heat treatment: mainly terpene hydrocarbons as well as dimethyl sulphide and some aliphatic aldehydes, and negatively correlated with the decreasing ones. The reverse was true for the pleasant odour qualities (60).

Other juice components than aroma compounds are affected by the processing of black currant berries to juice. 20-25 % of anthocyanins and 20% of ascorbic acid are lost (57,111), and the content of lipid, protein and carbohydrate is reduced (see Table 1, p. 3). Most of the hemicellulytic sugars and cellulose can be found back in the press cake (112). In addition, browning reactions occur (12).

5.1.2 Thermal treatment of black currant juice

As will be discussed in Section 5.2.1 is the aroma profile of black currant juice altered during conventional juice concentration. In order to determine the extent to which the application of heat in the concentration process is responsible for these changes, a study of thermal treatment of black currant juice was conducted (paper IV).

The changes in aroma compounds of black currant juice heated at 45, 60, 75 or 90°C for up to 60 minutes were determined (paper IV). The compounds were largely unaffected by heating at 45 and 60°C, whereas heating at 75 °C for 30 and 60 minutes, and at 90°C for 2.5, 5, 10, 30 and 60 minutes resulted in aroma changes. Higher temperatures and longer exposure times had larger effects on the aroma compounds. The concentration of aldehydes, furans, phenols, and benzene derivatives increased, where as most esters and alcohols slightly decreased (outlined in Table 7).

The concentration of most terpenes increased, in particular the terpene hydrocarbons. The concentration of the terpene alcohols 4-terpineol and linalool decreased where as the concentration of α-terpineol increased. Triangle tests revealed that the odour of black currant juice heated at 60°C for 30 and 60 minutes did not significantly differ from that of the control juice, where as the odour of juice heated at 90°C for 2.5, 10, 30 and 60 minutes significantly differed from the odour of the control. Strongly heated samples were unsolicited characterized by the judges as boiled, sharp, off-flavoured, earthy, burned, and less black currant.

Table 7. Outline of aroma changes during thermal treatment and concentration of black currant juice.

Compound group	Thermal treatment (paper IV)	Conventional evaporation concentration (paper I)
Furans	+[a]	+
Benzene derivatives	+	+
Esters	-[b]	-
Alcohols	-	-
Terpene hydrocarbons	+	-
Terpene alcohols	+/-	-
Other oxygenated terpenes	+	+/-

[a] Increase in concentration. [b] Decrease in concentration.

Paper IV partly confirms results of (62), where heating of black currant juice at 70 and 85°C for 30 minutes resulted in increases of aldehydes, dimethyl sulphide, 2-methyl-3-buten-2-ol and benzene derivatives. On the contrary a decrease of myrcene and terpinolene was observed in that study, however the presence of terpene alcohols was not reported. Higher temperatures caused larger effects on aroma compounds in the juice, and juice heated in an open system lead to a decrease of most compounds.

In (56,61) short time heating of black currant juice at 80 or 88°C caused minor or no changes in the aroma compounds, except for an increase of terpinyl acetate. No significant sensory differences were found between samples in (61) either, however for some sensory parameters small but significant effects were observed in (113), namely increasing sweet and natural aroma, as well as black currant and natural flavour.

The thermally induced increase of benzyl alcohol, benzaldehyde, methyl salicylate, eugenol, cumin aldehyde, and linalool oxide observed in paper IV can at least partly be explained by release from glycosidic precursors (see Section 4.3, paper VII and VIII). An additional considerable number of aliphatic alcohols and some oxygenated terpenes were glycosidically bound (Section 4.3, paper VII and VIII), but did not increase during heating, implying that the applied heat does not induce a glycosidic release of these compounds.

The changes observed in the terpene complex by thermal treatment of black currant juice (paper IV) were further investigated in paper VII. Acid catalyzed reactions of limonene, α-terpinene, linalool, α-terpineol, 4-terpineol and menthol were studied in black currant juice and in a model system subject to 90°C thermal treatment for 30 minutes. Similar changes of the terpenes were observed in the two systems, and the thermal treatment led to a decrease of all the investigated

compounds (paper VII, table 2). Menthol was the most stable of the studied terpenes showing only minimal loss. 4-Terpineol and α-terpineol were degraded by 29 and 21 %, respectively. 1,4-cineole was formed from these compounds, and γ-terpinene was also formed from 4-terpineol. Limonene and linalool were degraded to similar extends, 69 and 62 % respectively. α-terpineol was formed from both (114), and nerol and geraniol was formed in addition from linalool (115) (see Figure 5, p. 16). α-Terpinene was subject to the most extensive degradation (92 %), but no conversion products were detected, and generally the loss of compounds exceeded the detected increases in concentrations of products formed.

1,8-*p*-menthanediol and other terpene diols are reported to form from α-terpineol and 4-terpineol by acid catalyzed hydration and further transformation to 1,8-cineole or 1,4-cineole (114,116-119). An undetected formation of terpene diols might explain the overall loss of products observed, as terpene diols might not be extracted in the ether/pentane phase as suggested by (119). Therefore the use of more polar solvents might lead to improved recovery of these compounds (120). Due to their hydrophilic nature terpene diols are probably not recovered by headspace sampling either. Limited information exists on the presence of terpene diols in black currant products. Limonene diols were identified in (7) mainly recovered in a juice concentrate, and *cis*-*p*-menth-2-ene-1,8-diol was reported in extract of buds (121).

Paper VII demonstrates that the increase of linalool oxide observed by thermal treatment of black currant juice in paper IV and in (7,47) can in addition to a possible glycosidic release originate from heat induced conversion of 4-terpineol or α-terpinene. Likewise, the increase in concentration of α-terpineol observed in paper IV and in (47) could be caused by acid catalyzed conversion of various terpenes. On the other hand, the decrease of linalool measured in paper IV and (56) may be explained by the rearrangement and cyclisation into other terpineols. The increase of *p*-cymene, γ-terpinene, α-terpinene and terpinolene detected in paper IV and (47,56) arise from degradation of other terpenes. In conclusion, the terpene changes observed during thermal treatment of an aqueous acidic system like black currant juice can be explained mainly by acid catalyzed terpene reactions and to a minor degree glycosidic release. The reactions are part of a complex system where compounds are formed and degraded simultaneously.

Diverse reactions are involved in the increasing concentration of other compounds resulting from thermal treatment. Furans, increasing drastically during heating, are derived from the thermal degradation and rearrangement of sugars and ascorbic acid (122,123), and β-damascenone is formed during the oxidative degradation of carotenoids (124). The concentration of some phenols can increase due to thermal degradation of phenolic carboxylic acids (125). Esters are lost due to acid hydrolysis.

5.2 Aroma changes during concentration of black currant juice

5.2.1 Conventional concentration

Aroma changes during concentration of black currant juice in a multi-step falling film evaporator system was studied in paper I, and recoveries of aroma in aroma distillate, juice concentrate and singlings were determined relative to the initial base juice. The juice concentrate contained only a small amount of the aroma constituents; only benzene and furan derivatives were recovered in amounts of more than 5% of the amount present in the base juice (paper I, table 1). The bulk of aroma compounds were recovered in the aroma distillate, but for several compounds less than 50% of the initial concentration was recovered in that fraction. In the singlings a significant amount of some aroma compounds were detected, in particular furan and benzene derivatives, as well as terpene carbonyls. Reconstituted juice, constituting aroma distillate and juice concentrate, was subject to major reductions of all alcohols, terpene alcohols and terpene oxides, and of most esters and terpene hydrocarbons (outlined in Table 7). On the contrary, recoveries exceeded 110% for some compounds. Taking into account the great amount of some compounds in the singlings, large amounts of furans, benzene derivatives and terpene carbonyls had been generated. The changes are expected to influence the sensory quality of the reconstituted juice with a decrease in fruity notes (see Section 4.2).

The results of paper I confirms the observations made by Kollmansberger and Berger (7) who compared the changes of volatiles in two separate industrial-scale processes of black currant juice concentration. Only discrepancies are the larger overall recoveries of some alcohols in their study.

The increase of aroma compounds observed in juice concentration (paper I) can be explained by the thermally induced changes discussed in paper IV and Section 5.1.2. On the contrary the reductions observed for alcohols, terpene alcohols and terpene oxides, and for most esters and terpene hydrocarbons are only to a limited extend explained by the influence of heat. This suggests that aroma changes during juice concentration are not only thermally induced but are influenced by other processing factors such as evaporation or loss through the vacuum pump of the evaporator unit (see Section 2.3.2). This is however not well documented.

5.2.2 Membrane distillation

The potential of VMD and SGMD techniques for the recovery of black currant aroma was evaluated in paper III and VI.

Paper III studied the recovery of seven characteristic aroma compounds in black currant juice (methyl butanoate, ethyl butanoate, 1,8-cineole, ethyl hexanoate, *cis*-3-hexen-1-ol, furfural and β-damascenone) by laboratory scale VMD carried out at temperatures of 10-45°C and varying feed flows. To be able to evaluate the aroma recovery the mass balance must be approximately 100%, and the mass balances of the aroma compounds in the process were satisfactory, ranging from 84-109%. Only at 45°C was the recovery of furfural 126%, indicating that this temperature facilitate furfural formation. The concentration factors (CF) (ratio between concentration in

permeate/concentration in feed) of the aroma compounds could be categorized into significantly different groups according to their volatility. High feed flow rate and low temperature gave the highest CF's. At a 5% feed volume reduction of the juice (10°C), the esters and 1,8-cineole was recovered at 68-83%, where as the recovery of the less volatile compounds β-damascenone and *cis*-3-hexen-ol were only 32-38%.

The influence of SGMD on recovery of 12 aroma compounds was examined in laboratory scale set-ups and compared to VMD (paper VI). A model aroma solution and black currant juice were used as feed. In all experiments the total recovered aroma was satisfactory, ranging from 89% to 110%. High feed flow rate, high temperature and high sweeping gas flow rate gave the highest CF. The levels at which the aroma was recovered (45°C) at a 13.5% feed volume reduction differed markedly for the aroma compounds, depending on their volatility. The highest recoveries were obtained for the highly volatile aroma compounds 1,8-cineole and the esters with CF of 72-83%, whereas the CF's were smaller for the less volatile aroma compounds 4-terpinenol (50%) and *cis*-3-hexen-1-ol (35%).

In SGMD the feed temperature employed was higher and the CF's obtained were generally lower than for VMD. It was the same aroma compounds, which in both techniques obtained the highest CF's depending on volatility, but the great variability in CF's express that the complexity of fruit aroma volatiles is also a problem in recovery in membrane distillation. Comparison of results of the SGMD technique with the VMD technique was difficult partly because the membrane module design used was different and partly because the rate of the sweeping gas flow used in the experimental set up was not optimal for the process. Overall the VMD technique appears to be the better MD method to concentrate black currant juice, and it is concluded that MD's have some potential for gentle concentration of thermally sensitive fruit juices. In order to obtain the desired °Brix of the juice concentrate it is suggested that when an acceptable amount of aroma has been recovered the temperature can be increased to remove water at much higher flux, or water can be removed by another technique, as part of an integrated process. A further concentration of the permeate is however also necessary to reduce the volume.

6. Conclusions and perspectives

In the present work issues connected with the influence of thermal treatment and concentration on black currant juice aroma were investigated, in addition to aspects associated with methods for analysis of the aroma compounds.

Heating of black currant juice at 90°C within the time range used in conventional evaporation of black currant juice was shown to affect the aroma and sensory properties. The concentration of aldehydes, furans, phenols, and benzene derivatives increased, where as most esters and alcohols slightly decreased. The concentration of most terpenes increased, except linalool and 4-terpineol, which decreased. Heating of black currant juice at 45°C as used in the membrane distillation processes did not induce thermal changes to black currant juice.

The closer investigation of the terpene changes caused by thermal treatment of the juice elucidated that selected terpenes were degraded and converted into other terpene hydrocarbons and oxygenated terpenes. However, the loss of compounds generally exceeded the concentrations of products formed in the experiments, suggesting that non-volatile compounds like terpene diols might have been formed. Nevertheless this remains to be confirmed. In conclusion, the terpene changes observed by thermal treatment of an aqueous acidic system like black currant juice can be explained mainly by acid catalyzed terpene reactions and to a minor extent by glycosidic release. The reactions are part of a complex system where compounds are formed and degraded simultaneously.

Conventional concentration of the juice influenced most of the aroma compounds identified. Relative to the base juice an increase of furfural and some benzene derivatives and carbonyls was seen. In contrast, significant reductions were seen in the reconstituted juice for the majority of the compounds, including alcohols, esters and the majority of the terpenes. Esters and oxygenated terpenes are important to black currant juice odour, hence the changes are expected to influence the sensory quality of the reconstituted juice with a decrease in fruity notes. The increase of the aroma compounds observed during juice concentration can be explained by the thermally induced changes demonstrated. On the contrary are the observed reductions only to a limited extent explained by the influence of heat. This suggests that aroma changes during juice concentration are not only thermally induced but are also influenced by other processing factors.

The membrane distillation processes were superior to the conventional evaporation concentration in that no overall processing losses of aroma were observed. For more volatile compounds a high recovery was obtained in the aroma distillate, but for less volatile compounds a low recovery was found. However, problems remaining to be solved, are that an increased °Brix of the juice concentrate is required as well as the aroma distillate needs further concentration.

Investigations of the adsorbent materials revealed that terpene alcohols are degraded on Tenax GR during headspace sampling. The adsorbent materials Tenax TA and GR have otherwise similar properties, hence it is concluded that Tenax TA is more suitable than Tenax GR for determination of aroma compounds in black currant juice.

Choice of aroma isolation technique was found to influence the results of GC-O in relative number and type of identified compounds as well as by order of perceived importance. Compounds with lower volatility were generally recovered by solvent extraction, than by the headspace methods.

In future work, investigation of loss of aroma compounds through the vacuum pump during conventional concentration as well as otherwise processing loss should be further investigated. Alternative solvents or techniques for identification of terpene diols that potentially are formed at elevated temperatures might also elucidate the cause for some of the terpene changes observed in the present investigations. From an aroma quality perspective the application of lower temperatures in industrial juice concentration might be advantageous, if feasible.

Sensory profiling analysis of ready to drink black currant products from concentrated and single strength juices is suggested, to evaluate particular differences in the sensory attributes. These can in terms be correlated to results of GC-O and taste component analysis aiming at selecting compounds as indicators of aroma quality. It remains to be established how the aroma profile of reconstituted juice made from a final membrane distillation concentrate relate to that of a conventionally concentrated product.

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Paper I

Aroma recovery during concentration of black currant juice

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Varming, C. and Poll, L.

Aroma recovery during concentration of black currant juice

Camilla Varming and Leif Poll

Department of Dairy and Food Science, The Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark.

Abstract

The scope of this work was industrial scale concentration of black currant juice. Aroma recoveries in each fraction of the process were determined by GC-MS, and impact compounds of the base juice were identified by GC-olfactometry. The bulk of juice aroma was recovered in the aroma distillate. The singlings were a significant source of aroma loss, and for some compounds an overall processing loss was also observed. In reconstituted juice there was a significant decrease of many compounds, but an increase of other heat induced compounds was also observed. Key odours in the base juice were found mainly to be esters and terpenoids, of which a significant reduction was generally seen. The observed changes in aroma composition are to be expected to influence the sensory impression of the reconstituted juice.

Introduction

In the juice industry, concentration of fruit juices is applied in order to reduce volume and stabilise the product. The conventional method of concentrating fruit juices is by evaporation of water. During this heat-process some thermally induced changes occur, which affects the flavour of the reconstituted juice (von Sydow and Karlsson 1971; Kollmansberger and Berger, 1994). The undesirable sensory changes have been correlated with a decrease in terpenes and an increase of dimethyl sulfide and aliphatic aldehydes (Karlsson-Ekström and von Sydow, 1973). The scope of this work was to examine the effect of industrial scale concentration of black currant juice. For each fraction of the concentration process, namely aroma distillate, singlings, and juice concentrate, recovery of aroma was determined. Furthermore impact compounds of the base juice were identified by GC-olfactometry.

Experimental

Materials

The black currant (*Ribes nigrum* L. var. Ben Lomond) juice samples were obtained from an industrial scale multi-step falling film evaporator system. The temperature range in the concentration unit was 92-107°C. Samples were collected from each step of the process: base juice (9°Brix), aroma distillate, singlings, and juice concentrate (74°Brix). The aroma distillate constituted approximately 0.55% of the base juice volume, the singlings 81%, and the juice concentrate 18.5%. Samples were stored at –15 °C until aroma analyses were carried out.

Dynamic headspace isolation of aroma compounds

Samples were thawed, and 150 g of base juice/juice concentrate/singlings, or 5 g of aroma distillate and 145 g of water was transferred to a 500 mL blue cap bottle with 1.00 mL of internal standard (50 µL/L 4-methyl-1-pentanol). Under magnetic stirring (200 rpm) samples were

purged with nitrogen (100 mL/min) in a water bath at 30 °C for 60 min. Volatiles were collected on Tenax-GR traps.

GC-MS analysis

Separation and identification of aroma compounds was carried out on a Hewlett-Packard (Palo Alto, CA) G1800A System (GC-MS) equipped with a J & W Scientific DB-Wax column (30 m x 0.25 mm i.d., 0.25 µm) using helium as carrier gas. The volatiles were thermally desorbed from the traps using an ATD 400 Automated Thermal Desorber (Perkin Elmer, Norwalk, USA). The GC-MS split ratio was 1:10. Temperature programme: 10 min at 40°C, increase to 240°C at 6 °C/min, isothermal for 25 min. Samples were analysed in triplicate. Identification was carried out by probability-based matching with mass spectra in G1035A Wiley Library (Hewlett-Packard, Palo Alto, CA) and with mass spectra obtained in the laboratory from reference compounds. GC peak areas were calculated on the basis of single ions.

GC-olfactometry

GC-olfactomery and GC-FID (Flame Ionisation Detector) was carried out on a Hewlett-Packard 5890 GC equipped with a SGC Olfactory Detector Outlet ODO-1. The volatiles were thermally desorbed from the traps using a Short Path Themal Desorber Model TD-4 (Scientific Instrument Services, Inc., Ringoes, NJ, USA). The column and GC-settings were the same as for GC-MS; and for GC-olfactometry the FID was detached. Three assessors, who were trained in performing GC-olfactometry, sniffed the base juice isolate once by Odour Profiling Technique. The judges noted starting and ending time, description of odour quality, and intensity of the odour on a scale from 1 to 4. Each sniffing session continued for 40 min. Odours detected by one judge only were considered as noise. Impact compounds were selected on the basis of match between GC-olfactory and GC-MS retention times, and accordance with description of odours of the compounds found in the literature (Burdock, 1994).

Results and discussion

In table 1 is listed the aroma compounds identified in the black currant juice samples. Results are given as %-recoveries of the individual aroma compounds in the different fractions of the evaporation process, relative to the initial base juice content, which was 100%. The content of an aroma compound in the reconstituted juice is the calculated sum of the recovery in the aroma distillate and the juice concentrate, respectively.

Only compounds with a match between mass spectra of minimum 90, which could be separated from other peaks are included in the table. Coefficient of variation of the aroma analyses was within 10%.

Except for furfural, the juice concentrate contained very little aroma, hence the bulk of aroma was recovered in the aroma distillate. The singlings were a significant source of aroma loss for some compounds, where the greatest loss was seen for the furans. For the majority of the terpene alcohols and terpene carbonyls, and for some alcohols, benzene derivatives and 2-methyl-2-butenal significant amounts were also lost. For esters and terpene hydrocarbons (except *p*-cymenyl) there was no significant loss through the singlings. In addition an overall processing loss of some compounds occurred, which could be due to their break down or loss through the vacuum pump.

Table 1. % recoveries relative to base juice (100%) of aroma compounds identified in different fractions of the black currant juice evaporation process. Compounds written in italic are odour active compounds determined by GC-olfactometry.

	Aroma distillate	*Singlings*	*Juice conc.*	*Reconstit. juice*[a)]		*Aroma distillate*	*Singlings*	*Juice conc.*	*Reconstit. juice*[a)]
Terpene hydrocar.					2/3-methyl butanol	18	17	0	18
α-terpinene	50	3	0	50	1-pentanol	42	17	0	42
γ-terpinene	48	2	0	48	1-hexanol	50	9	1	50
terpinolene	35	1	0	35	*cis-3-hexen-1-ol*	*49*	*17*	*0*	*49*
p-cymen	32	3	0	32	trans-2-hexen-1-ol	58	15	0	58
p-cymenyl	101	29	4	105	1-octen-3-ol	55	4	0	55
α-phellandrene	66	0	0	66	1-heptanol	42	6	0	42
Terpene esters					2-ethyl-1-hexanol	23	3	1	25
bornyl acetate	64	1	0	64	1-octanol	67	8	2	70
citronellol acetate	83	0	0	83	1-nonanol	76	0	0	76
Terpene oxides					**Carbonyls**				
isocineol	48	0	0	48	2-met 2-butenal	101	47	1	102
Cineole	*34*	*2*	*0*	*34*	3-octanone	76	0	0	76
rose oxid	53	1	0	53	**Esters**				
vitispirane	57	3	0	57	*met butanoate*	*59*	*6*	*0*	*59*
Terpene alcohols					*ethyl butanoate*	*59*	*3*	*0*	*60*
thymol	*58*	*35*	*0*	*58*	butyl acetate	71	2	0	71
linalool	*64*	*5*	*3*	*67*	*isoamyl acetate*	*68*	*0*	*0*	*68*
4-terpineol	*57*	*8*	*0*	*57*	met crotanoate	108	7	0	108
1,8-menthadien-4-ol	70	18	0	70	ethyl crotanoate	121	2	0	121
α-terpineol	70	16	1	71	*met hexanoate*	*98*	*3*	*0*	*98*
p-cymen-8-ol	74	45	0	74	*ethyl hexanoate*	*67*	*1*	*0*	*67*
Terpene carbonyls					hexyl acetate	83	1	5	88
α-cyclocitral	98	6	0	98	met-E-2-hexenoate	74	0	0	74
1,4-dimet-3-cyclo-hexenylmethylketon	84	7	0	84	2-hexenyl acetate	104	0	0	104
pinocarvone	111	11	0	111	**Benzene derivatives**				
p-mentha-9-al	53	40	1	54	benzaldehyde	183	51	7	190
β-cyclocitral	75	7	0	75	ethyl benzoate	82	8	0	82
safranal	78	0	0	78	met salicylate	104	13	1	105
phellandral	113	15	0	113	ethyl salicylate	101	7	0	101
carvone	80	30	0	80	phenol	88	65	9	96
cumin aldehyde	76	34	2	78	**Furans**				
2-caren-10-al	170	20	0	170	furfural	165	145	33	198
damascenone	*103*	*7*	*0*	*103*	2-acetylfuran	88	104	9	97
Alcohols					met furoate	99	61	7	106
1-butanol	16	36	0	17	furfuryl alcohol	15	94	7	22

a) Reconstituted juice comprises the calculated sum of the aroma distillate and the juice concentrate.

Relative to the base juice, significant reductions were seen in the reconstituted juice for all alcohols, terpene alcohols and terpene oxides, and for most esters and terpene hydrocarbons. On the contrary, recoveries exceeded 110% for some terpene carbonyls, benzaldehyde, ethyl crotanoate and furfural. Taking into account the great loss of some compounds into the singlings, even more of the furans, benzene derivatives and terpene carbonyls have been generated. The furan derivatives are known products of heat induced sugar-acid reactions. Other compounds might have existed in glycosidically or otherwise bound forms in the juice, where thermal treatment of the acidic juice may accelerate their liberation. Moreover rearrangements and oxidation of terpenes has occurred during the concentration process.

During heating of black currant berries, von Südow and Karlsson (1971) also observed that furan derivatives and aldehydes were formed. They also found that most monoterpene hydrocarbons decreased, whereas the quantity of monoterpene alcohols increased.

Kollmansberger and Berger (1994) investigated multi-step evaporation of black currant juice. They observed a formation of furan derivatives, and losses of esters and cineole. Additionally a larger mass was recovered than was contained in the base juice for heat induced heterocycles, benzaldehyde, and benzoic and aliphatic alcohols.

Of the odours observed in the base juice by GC-olfactometry (table 1, *italic*), the majority of the eleven impact compounds which could be identified were esters and terpenoids. By Iversen *et al*. (1998) and Latrasse *et al*. (1982) the compounds methyl butanoate, ethyl butanoate, ethyl hexanoate, cineole, linalool, 4-terpineol and damascenone have previously been reported to be of importance for the flavour of black currant berry and/or nectar.

Recovery of impact compounds in reconstituted juice was approx. 100% for damascenone and methyl hexanoate, while a decrease of 34-68% was seen for the terpene alcohols, cineole, cis-3-hexen-1-ol and the esters (except methyl hexanoate). This reduction must be expected to influence the sensory quality of the reconstituted juice with a decrease in fruity, flowery and green notes. Further more the increase of compounds like furans and benzaldehyde during the concentration process is also likely to contribute to changes in the sensory impression of reconstituted juice. Karlsson-Ekström and von Sydow (1973) reported that unpleasant odour qualities were positively correlated with the compounds increasing with heat treatment, and negatively correlated with the decreasing compounds.

Conclusion

During the concentration of black currant juice, the bulk of aroma was recovered in the aroma distillate. The singlings were a significant source of aroma loss, and for some compounds an overall processing loss was also seen. Relative to the base juice, significant reductions were seen in the reconstituted juice for all alcohols, terpene alcohols and terpene oxides, and for most esters and terpene hydrocarbons. On the contrary, an increase of furfural, benzaldehyde and some carbonyls was seen.

The key odours in the base juice were mainly found to be esters and terpenoids, of which a significant reduction was generally seen during the concentration process. This is to be expected to influence the sensory impression of the reconstituted juice.

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Paper II

Comparison of isolation methods for the determination of important aroma compounds in black currant (*Ribes nigrum* L.) juice, using NIF profiling

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Comparison of Isolation Methods for the Determination of Important Aroma Compounds in Black Currant (*Ribes nigrum* L.) Juice, Using Nasal Impact Frequency Profiling

CAMILLA VARMING, MIKAEL A. PETERSEN, AND LEIF POLL*

Department of Dairy and Food Science, Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

The influence of isolation method on the determination of important aroma compounds in black currant juice was investigated by surface of nasal impact frequency (SNIF) gas chromatography−olfactometry (GC-O). The applied methods were solvent extraction, static headspace, and purge and trap using 15 and 60 min of purge time. By the four methods, a total of 59 odors were observed, and, of these, 44 corresponded to compounds that could be identified. For the headspace methods increasing purge volumes resulted in recoveries of additional, less volatile compounds. The main compound groups recovered by the headspace methods were esters and terpenes, whereas compounds recovered by solvent extraction were not as dominated by fruity odors. For most compounds there was agreement between the size of the SNIF value obtained by GC-O and the amount of the measurable compound found by gas chromatography−mass spectrometry.

KEYWORDS: GC−olfactometry; NIF; SNIF; aroma isolation method; black currant juice aroma; GC-MS

INTRODUCTION

Most cultivation and usage of black currants occurs in Europe, and the main part of the fruit is processed as frozen berries, juice, syrup, or jam. Black currants are characterized by having high contents of vitamin C, organic acids, and anthocyanins (*1*). The aroma of black currant berries constitutes >150 aroma compounds, of which the major groups are terpenes, esters, and alcohols (*2*). The processing of berries to juice leads to some major changes in the aroma composition (*3*−*6*). Important compounds of black currant berries have been identified by gas chromatography−olfactometry (GC-O) by Latrasse et al. (*7*) and Mikkelsen and Poll (*5*) and those of black currant nectar and juice by Iversen et al. (*3*) and Varming and Poll (*6*). Compounds reported in two or more of these papers include methyl butanoate, ethyl butanoate, ethyl hexanoate, cineole, linalool, 4-terpineol, β-damascenone, 1-octen-3-one, 2-methoxy-3-isopropylpyrazine, and 4-methoxy-2-methyl-2-butanethiol.

Most aroma isolation methods are based on the analysis of either a solvent extract of a food or the headspace above it. During GC analysis of a solvent extract low-boiling compounds can be masked by the solvent front, and the method results mainly in the isolation of intermediate and higher boiling compounds. By static headspace collection, equilibrium between the sample and the headspace above it is obtained and usually a fraction of the headspace is withdrawn for GC analysis. During dynamic headspace collection and purge and trap the sample is purged with a gas stream above or through the sample, respectively, continuously removing the headspace, shifting the sample/air equilibrium. Reviews concerned with the issue of aroma isolation methods have been published (*8*, *9*).

The part of the volatiles present in a food system that is responsible for its odor can be identified by sniffing the GC effluent of an aroma isolate. Combined hedonic aroma response measurement (CHARM) analysis and aroma extract dilution analysis (AEDA) are GC-O methods that have often been used. These methods are based on one or a few assessors sniffing stepwise dilutions of a solvent extract until no odors can be detected. Methods of GC-O have been reviewed by Blank (*10*).

The principle of dilution to detection threshold has been questioned as the method is based on the assumption that slopes of the psychophysical functions for all aroma compounds are equal, which is not the case (*11*). Also, the occurrence of gaps in coincident response for panelists during extract dilution sniffing analysis has been contemplated (*12*), and due to large variances among subjects, more than one or a few assessors are required for reliable GC-O results (*11*−*14*). Some of these problems are overcome by the nasal impact frequency (NIF) method described by Linssen et al. (*15*) and Pollien et al. (*14*). The NIF method uses only one dilution level, but GC-O is repeated by a number of panelists; that is, data treatment is based on detection frequency rather than successive dilutions. For this method a panel of a minimum of 6, optimally 8−10, assessors

* Author to whom correspondence should be addressed [telephone (+45)-35283240; fax (+45)35283265; e-mail lep@kvl.dk].

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are needed for reliable results (*14*), and the method has been found to correlate well with sensory odor intensities (*13*).

The purpose of the present study was to determine important aroma compounds in black currant juice using four different isolation methods and to investigate how the relative importance of the aroma compounds is influenced by isolation method. Methods of solvent extraction, static headspace collection, and purge and trap were applied and important aroma compounds determined by NIF using nine assessors.

MATERIALS AND METHODS

Materials. A commercial black currant juice of the variety Ben Lemond was obtained from an industrial plant. The juice preparation included crushing, heating, enzyme treatment (50 °C/maximum 6 h), pressing, pasteurization (98 °C/30 s), clarification (45 °C/maximum 6 h), and filtration. The juice was stored at −18 °C and thawed immediately before use.

Static Headspace Collection. One hundred and fifty grams of black currant juice was weighed into a 500 mL glass flask equipped with a purge head. To maintain static conditions, the gas inlet of the purge head was sealed with a box nut. One milliliter of internal standard (50 μL/L 4-methyl-1-pentanol, Aldrich, Steinheim, Germany) was added to verify that the analysis performed satisfactory. The sample was placed in a water bath at 30 °C and allowed to equilibrate for 60 min. The box nut was then removed from the purge head and replaced with a tube connected to running tap water, slowly displacing (5 min) the headspace (430 mL) above the sample. The volatiles were collected on a trap containing 250 mg of Tenax-GR (mesh size = 60/80, Buchem bv, Apeldoorn, The Netherlands). To detect a possible breakthrough of volatiles, a second identical trap was connected in series with the first trap and subjected to GC-MS analysis.

Purge and Trap. Sample preparation was the same as for static headspace collection. The sample temperature was equilibrated in a 30 °C water bath for 10 min. Under magnetic stirring (200 rpm) the sample was then purged through the liquid with nitrogen (100 mL/min) for either 15 or 60 min, and volatiles were collected into Tenax GR traps. The purge times corresponded to purge volumes of 1.5 and 6.0 L, respectively.

Solvent Extraction. One hundred and fifty grams of black currant juice was weighed into a 500 mL blue-cap flask, and 50 mL of ether/pentane 1:1 and 1.00 mL of internal standard (50 μL/L 4-methyl-1-pentanol, Aldrich) were added. Volatiles were extracted for 30 min under magnetic stirring (100 rpm). The sample was then left for phase separation for 15 min and placed in a freezer, allowing the water phase to freeze and the solvent phase to be decanted. The solvent phase was then dried with Na_2SO_4 and concentrated to 0.20 g under a gentle stream of nitrogen. The extract was stored at −18 °C, and prior to GC analysis, 2.0 μL of the extract was injected into a Tenax-GR trap.

GC-MS. The collected volatiles were thermally desorbed using an automated thermal desorber (ATD 400, Perkin-Elmer). Desorption time from the trap (250 °C) to the cold trap (5 °C) was 15 min, with a helium flow of 60 mL/min. Volatiles were desorbed from the cold trap to the GC column by flash heating from 5 to 300 °C. Using a split ratio of 1:10, separation and identification of aroma compounds was carried out on a Hewlett-Packard (Palo Alto, CA) G1800A S GC-MS system equipped with a J&W Scientific DB-Wax column (30 m × 0.25 mm × 0.25 μm) using helium as carrier gas (1 mL/min). The column temperature was kept at 40 °C for 10 min, increased with 6 °C/min to 240 °C, and kept isothermal for 25 min. For analysis of solvent extracts, a solvent delay of 3 min was used. The mass selective detector used the electron ionization mode, and the mass/charge (*m*/*z*) range between 20 and 425 was scanned. Samples were analyzed in triplicate. Identifications were carried out by probability-based matching with mass spectra in the G1035A Wiley library (Hewlett-Packard) and comparisons with mass spectra and retention indices (RI) of authentic reference standards analyzed under identical conditions. Aroma standards (numbers referr to **Table 1**) were obtained from **1**, **3**, **6-1**, **10**, **12**, **13**, **16**, **19**, **21**, **23**, **25**, **27**, **29**, **31**−**33**, **35**, **36**, **40**, **50**, **51**, and **58** (Sigma-Aldrich, Copenhagen, Denmark), **2**, **4**, **5-2**, **7**, **8**, **14**, **49**, and **57** (Merck, Darmstadt, Germany), **5-1**, **6-2**, **20**, and **30** (Fluka, Buchs, Switzerland), **55** (Supelco, Bellefonte, PA), **34** (Roth, Karlsruhe, Germany), **17** (Lancaster, Morecambe, U.K.), 15 (K&K Laboratories, Plainview, NY), and **46** (Firmenich, La Plaine, Switzerland). When authentic reference standards could not be obtained, tentative identifications were based on matching with mass spectra in the Wiley library and comparisons of RI and odor properties reported in the literature. Linear retention indices were calculated after analysis under the same conditions of an *n*-alkane series (C9−C24). The amounts of the measurable identified aroma compounds were calculated on the basis of single ions.

GC-O. GC-O and GC−flame ionization detection (FID) were carried out on a Hewlett-Packard 5890 GC equipped with an SGE olfactory detector outlet ODO-1. The volatiles were thermally desorbed from the traps using a short-path thermal desorber model TD-4 (Scientific Instrument Services, Inc., Ringoes, NJ). Dry purge time was 20 min, and desorption time was 3 min. The column type and GC settings were the same as for GC-MS. For GC-O the column was detached from the FID and led directly to the sniffing port, where the effluent was mixed with humidified air (150 mL/min). Nine people between 25 and 61 years of age were recruited among staff and students of the department. The panelists, who all were familiar with GC-O, were instructed to note starting and ending time of the odors and to give free choice descriptions of the odor qualities. One sniffing session continued for 40 min, and each panelist participated once in the sniffing of each of the four isolates, performed in random order. The nine individual profiles were summed to one NIF profile, but odors detected by only one or two judges were considered to be noise (*13*). Peak heights (number of judges) of the profiles are termed nasal impact frequency (NIF), and peak areas are termed surface of nasal impact frequency (SNIF) (number of judges × min).

Pollien et al. (*14*) estimated a least significant difference (LSD) between SNIF values for a given peak found in two different samples. The LSD was calculated from the standard deviation (SD), based on between- and within-panel variation, and Student's constant (*t*), which takes into account the number of degrees of freedom:

$$\mathrm{LSD} = t(\sqrt{2})\mathrm{SD} \quad (1)$$

We used this approach, as an approximation, to estimate the LSD between SNIF values of the four isolation methods of a given peak. With the number of peaks in our study, *t* approaches 2, and the SD is based on an average relative standard deviation of 18% (*18*) of the SNIF mean value for a given peak:

$$\mathrm{LSD} = 2(\sqrt{2})18/100 \times \bar{x} \quad (2)$$

GC-O/FID retention times were correlated to GC-MS retention times using a standard mixture of potent aroma compounds in the relevant retention time span, analyzed under the same conditions.

RESULTS AND DISCUSSION

Odors Observed and Compounds Identified by the Four Methods. A total of 59 contributors to the aroma were detected by GC-O by three or more judges. Of these, 44 corresponded to compounds that could be identified either fully or tentatively (**Table 1**). The remaining 15 compounds were present in concentrations below the GC-MS detection limit but above the sensory threshold of GC-O, and they were all in the medium- to low-volatility area. The main groups of compounds identified were esters and oxygenated terpenes. Compared to earlier studies of important compounds of black currant berry or juice, the total number of odors observed in this study was much higher. This is explained by (1) the application of four methods covering a broad spectrum of compounds, (2) a purge time of 60 min with a flow of 100 mL/min, which allowed a considerable concentration of aroma compounds, and (3) participation of nine assessors, which increased the sensitivity of GC-O. Compounds identified in this study that were previously reported by two or

Table 1. Odor Descriptors of Black Currant Juice Aroma Compounds As Determined by Each of the Four Isolation Methods

				SNIF[c] values					
no.	RI[a]	compound	odor descriptors[b]	static headspace	P&T 15	P&T 60	solvent extraction	LSD[d]	lit.[e]
1	844	dimethyl sulfide[f]	vegetable soup, cabbage, moldy	1.9	1.4	0	m[g]	0.6	
2	864	methyl acetate[f]	fruit, solvent, black currant juice	2.3	2.3	1.0	m	1.0	
3	916	methyl 2-methylpropanoate[f]	fruit	0	0	0.4	m	0.1	
4	947	ethyl propanoate[f]	solvent, acetone, fruit	1.2	2.6	2.4	m	1.1	
5	965	2,3-butanedione[f]	caramel, dirty socks, fruit, spirit, pineapple	3.5	4.2	5.9	1.4	1.9	*7*
	989	methyl butanoate[fh]							*3, 5, 6*
	1004	methyl 2-methylbutanoate[f]	fruit, spirit, solvent, chewing gum	2.9	2.9	2.2	0.8	1.1	
6		*and/or*							
	1007	2-methylpropyl acetate[f]							
7	1030	ethyl butanoate[f]	fruit, pineapple, acetone, caramel	4.8	4.3	5.6	3.9	2.4	*3, 5–7*
8	1055	ethyl 3-methylbutanoate[f]	black currant, sweet, acidulous fruit	0	0	0.9	0	0.1	
9	1094	methyl *trans*-2-butenoate[i]	black currant, fruit	0	0	1.5	0	0.0	
10	1115	mixture of 2- and 3-methylbutyl acetates[f]	banana, chewing gum	0	0	0.9	0	0.1	*6*
11	1171	isocineole[f]	solvent, sweet, flower	0.8	0.8	1.3	0	0.4	
12	1194	cineole[f]	liquorices, menthol, pine, cat urine	0.8	1.9	3.0	0	0.6	*5–7*
13	1206	mixture of 2- and 3-methyl-1-butanols[f]	sweat, green, acidulous, fruit	0	1.4	2.8	1.5	0.7	*5, 7*
14	1229	ethyl hexanoate[f]	fruit, wine gum, sweets	2.1	2.1	1.6	0.3	0.8	*3, 5, 6*
15	1267	hexyl acetate[f]	tobacco, acidulous, citrus, green, herbs	0	0	1.1	0.3	0.2	
16	1278	octanal[f]	flower, fruit, orange	0	0	1.0	0	0.1	
17	1289	1-octen-3-one[f]	mushroom	0.7	1.5	1.4	0.8	0.6	*3, 5*
18	1305	2-methyl-3-furanthiol[i]	vitamin, bouillon, cooked meat	0.3	1.9	3.4	2.4	1.0	
19	1313	6-methyl-5-hepten-2-one[f]	black currant, boiled fruit, "bitter"	0	0	1.3	0	0.2	
20	1332	*cis*-rose oxide[f]	paper, flower, greenish	0.4	0.4	1.6	0	0.3	*5*
	1361	methyl-2-hydroxy butyrate[k]	flower, yeasty, deep frying fat,	1.6	2.1	1.7	1.2	0.8	*6*
21		*and/or*	spoiled fruit						
	1372	*cis*-3-hexen-1-ol[f]							
22	1378	methyl octanoate[f]	green	0	0	0.4	0	0.1	*3*
23	1380	nonanal[f]	library, flower, citrus	0.7	0.7	0.7	0	0.3	*3*
24	1398	unknown	mushroom, sour dishcloth	0	0	0.4	0.7	0.1	
25	1419	2-methoxy-3-isopropylpyrazine[f]	pea, dry, pea pod, grass, bell pepper	1.2	1.2	1.8	1.2	0.7	*5, 7*
26	1429	acetic acid[f]	acetic acid	0	0	0	0.7	0.1	
27	1433	methional[f]	boiled potato, deep frying fat	1.0	1.2	2.0	2.0	0.8	
28	1469	unknown	unpleasant flower, deep frying fat	0	0	0.7	0	0.1	
29	1476	decanal[f]	sweetish, orange, flower	0	0	0.7	0	0.1	
	1498	camphor[f]	green, dry, green house, leaf	0	0.8	1.3	0.5	0.3	
30		*and/or*							
	1504	1,4-dimethyl-3-cyclohexenylmethyl ketone[k]							
31	1512	3-methoxy-2-isobutylpyrazine[f]	dry, green, leaf, spicy, green pepper	0.7	1.3	1.6	0.6	0.5	
32	1530	(*E*)-2-nonenal[f]	library, cucumber salad	0	0.4	1.1	0.2	0.2	*5*
33	1540	linalool[f]	althea, flower, sweet candy, fruit	0	0.5	1.2	0	0.2	*6, 7*
34	1595	4-terpineol[f]	green, licorices, moldy	0	0	1.4	0	0.2	*5, 6*
35	1613	butanoic acid[f]	parmesan cheese, vomit, butanoic acid	0	0	0	2.1	0.3	
36	1655	mixture of 2- and 3-methylbutyric acids[f]	dirty socks, vomit, butanoic acid	0	0	0	1.4	0.2	
37	1658	3-mercapto-3-methyl-1-butanol[i]	bouillon, vitamin, cooked meat	0.7	1.1	1.1	0	0.4	
38	1668	unknown	sour, flower, pearl onion	0	0	0.5	0	0.1	
39	1679	unknown	spoiled food	0	0	0.7	0	0.1	
40	1688	α-terpineol[f]	rice, green, flower, washing up liquid	0	0.2	0.4	0.6	0.2	
41	1710	phellandral[k]	rice, licorices, flower	0	1.1	2.8	1.4	0.7	
42	1716	unknown	liver pate, onion	0	0	0	0.9	0.1	
43	1754	unknown	green, cucumber, acid, dry	0	0	0.6	0	0.1	
44	1785	unknown	rice, greenish, moldy, tobacco	0	0	0.4	0.5	0.1	
45	1801	unknown	boiled fruit, alcoholic fruit, flower	0	0	1.0	0	0.1	
46	1813	β-damascenone[f]	black currant juice, boiled fruit, flower	0.9	1.0	1.3	1.1	0.5	*5–7*
47	1838	unknown	tobacco, woody, rubber	0	0	1.2	0.6	0.2	
48	1860	unknown	baked oats, fruit juice	0	0.4	0	0	0.1	
49	1866	benzyl alcohol[f]	perfume, flower, fruit	0	0	0.4	0	0.1	
50	1902	2-phenylethanol[f]	flower, rose	0	0	1.0	0.9	0.2	
51	1936	β-ionone[f]	flower, rose, menthol, berries, violet	0	0.6	0.5	0	0.1	
52	1991	unknown	curry, licorices	0	0	0	0.5	0.1	
53	2004	unknown	flower	0	0	0.5	0	0.1	
54	2027	unknown	clove, cake, spices	0	0	1.0	0.7	0.2	
55	2068	4-methylphenol[f]	bad smell, horse manure, leather	0	0	0.9	0.8	0.2	
56	2131	unknown	fruit, flower, scent eraser	0.7	1.2	1.5	1.4	0.6	
57	2153	eugenol[f]	Christmas, dentist, eugenol	0	0	1.7	1.0	0.3	*5*
58	2181	4-vinyl-2-methoxyphenol[f]	dentist	0	0	0	0.6	0.1	
59	2220	unknown	coconut, mandarin, dill, perfume	0	0	0.8	2.7	0.4	

[a] Retention indices calculated from GC-MS data. [b] Most frequent odor quality perceived during GC-O. [c] Peak areas of individual odors detected by three to nine assessors. [d] Estimated from eq 2. [e] Compounds previously reported as being important in black currant berry or juice. [f] Mass spectra and RI agreed with authentic standards. [g] Sniffing started at RI 950 due to the solvent peak. [h] Odor descriptions matching methyl butanoate were only recorded for P&T 60 and were here mixed in the descriptions of 2,3-butanedione. [i] Mass spectra agreed with the Wiley library and RI agreed with literature values. [j] No interpretable MS signal; RI and aroma properties agreed with the literature. [k] Mass spectra agreed with the Wiley library. [l] No interpretable MS signal; RI and aroma properties agreed with authentic standards.

Table 2. Top Five Most Potent Compounds of Black Currant Juice Aroma As Determined by Each of the Four Isolation Methods

static headspace		P&T 15		P&T 60		solvent extraction	
SNIF[a]	compound	SNIF	compound	SNIF	compound	SNIF	compound
4.8	ethyl butanoate (**6**)[b]	4.3	ethyl butanoate (**6**)	5.9	2,3-butanedione (**5**) methyl butanoate	3.9	ethyl butanoate (**6**)
3.5	2,3-butanedione (**5**)	4.2	2,3-butanedione (**5**)	5.6	ethyl butanoate (**6**)	2.7	unknown (**59**)
2.9	methyl 2-methylbutanoate 2-methylpropyl acetate (**6**)	2.9	methyl 2-methylbutanoate 2-methylpropyl acetate (**6**)	3.4	2-methyl-3-furanthiol (**18**)	2.4	2-methyl-3-furanthiol (**18**)
2.3	methyl acetate (**2**)	2.6	ethyl propanoate (**4**)	3.0	cineole (**12**)	2.1	butanoic acid (**35**)
2.1	ethyl hexanoate (**14**)	2.3	methyl acetate (**2**)	2.8	2/3-methyl-1-butanol (**13**)	2.0	methional (**27**)

[a] SNIF = peak areas of individual odors. [b] Numbers in parentheses correspond to compounds in **Table 1**.

more papers, by GC-O, as important for black currant berry or juice are methyl butanoate, ethyl butanoate, cineole, 3-methyl-1-butanol, ethyl hexanoate, 1-octen-3-one, 2-methoxy-3-isopropylpyrazine, linalool, 4-terpineol, and β-damascenone. Other compounds previously reported as being important for black currant aroma were, however, not found to be important in the present study (*3*, *5*−*7*). This could be due to different isolation and GC-O methods being used, as well as berry variety and degree of processing influences on the aroma profile (*4*−*6*, *16*). Some of the compounds identified by GC-MS in this study have not previously been reported in either black currant berry or juice. With varying certainty of identification these compounds were methyl 2-methylpropanoate, ethyl propanoate, 2-methyl-3-furanthiol, 6-methyl-5-hepten-2-one, methional, 3-methoxy-2-isobutylpyrazine, 3-mercapto-3-methyl-1-butanol, and 4-vinyl-2-methoxyphenol.

The most odors were observed by P&T 60 (51) followed by solvent extraction (32), P&T 15 (28), and static headspace (20). This was expected because P&T 15 results in collection of 3.5 times the headspace volume of static headspace, and P&T 60 results in collection of 4 times the headspace volume of P&T 15. P&T 60 and solvent extraction led to relatively more odors with high retention indices than static headspace and P&T 15. For most odors, P&T 60 resulted in the same or higher SNIF values than the other methods. Exceptions from this were compounds with retention indices below 900, where smaller SNIF values were observed by P&T 60 due to breakthrough on the Tenax GR traps. When solvent extracts were analyzed, only a fraction of the extract could be injected; therefore, SNIF values of this method were sometimes smaller than for P&T 15 and P&T 60.

Six odors corresponding to less volatile compounds were observed only by solvent extraction, namely, the three acids, 4-vinyl-2-methoxyphenol, and two unknowns. Sixteen odors were observed by P&T 60 only, namely, five esters, three carbonyls, one alcohol, one terpene, and six unknowns. Comparing the headspace methods, all odors perceived by static headspace collection were also observed by P&T 15, and all odors observed by P&T 15 were also observed by P&T 60. The only exceptions were dimethyl sulfide, due to breakthrough of the traps, and an unknown (**48**). Static headspace collection and P&T 15 differed from P&T 60 particularly in that a lower number of esters and aldehydes, and no phenolics, were observed. The relative number of esters and terpenes observed by solvent extraction was lower than for the headspace methods.

A ranking of compounds within each method according to their SNIF values (based on **Table 1**) is shown in **Table 2**. Ethyl butanoate was the only compound ranked in the top five by all methods. 2,3-Butanedione was represented by the three headspace methods, and static headspace and P&T 15 further ranked three esters in the top five, of which two were the same. According to P&T 60 and solvent extraction no additional esters were in top five, but both ranked 2-methyl-3-furanthiol in the five most important. One terpene and one alcohol were further ranked by P&T 60. When compared to the other headspace methods P&T 60 ranked some less volatile compounds in the top five. Compounds additionally ranked by solvent extraction were butanoic acid, methional, and an unknown. Relative to the headspace methods solvent extraction was dominated by fewer compounds representing fruity odors.

Partially confirmatory results have been reported for the GC-O analysis of cooked seafood products. Purge and trap and static headspace were found to give similar results for the more volatile compounds, whereas AEDA of solvent extraction methods was characterized by identification of mainly intermediate- and low-volatility compounds (*17*, *18*). In a study concerning tea powder, the majority of the compounds identified by static headspace were also identified by AEDA, but by AEDA several additional compounds were identified (*19*).

Relative Concentrations of Compounds Identified by the Four Methods. The relative concentrations of measurable odorous compounds are shown in **Table 3**. Results are based on MS peak areas of single ions, where the highest concentration measured of each compound is set to 100. Some of the compounds listed for static headspace, P&T 15, and solvent extraction were present in very low concentrations; hence, identification was only possible using P&T 60. P&T 60 gave the best results in terms of aroma recovery, followed by P&T 15, but for some of the less volatile compounds the largest amounts were recovered by solvent extraction. The observation by GC-O (**Table 1**) that P&T 60 was subject to breakthrough of the traps for dimethyl sulfide and methyl acetate, and P&T 15 for dimethyl sulfide, was verified by GC-MS. By GC-O, 10 known compounds were detected only using P&T 60 (**Table 1**), whereas by GC-MS decanal and phellandral were the only two compounds detected solely by P&T 60, meaning that the concentrations obtained by the other methods for the remaining eight compounds must have been lower than their odor thresholds.

Generally SNIF values (**Table 1**) corresponded to GC-MS peak areas. The few deviations from this can be due to assessors being less sensitive to changes in concentration than GC-MS or the concentration level having reached the assessor's response plateau. In addition, broader and more overlapping peaks were found by GC-O of P&T 60 than by the other headspace methods; hence, SNIF values of the involved coeluting aroma compounds may be uncertain. In a study by Pollien et al. (*20*) some, but not high, correlations between SNIF values and concentrations of a standard aroma solution were found.

Determination of the isolation method resulting in the GC-O profile closest to the situation during eating can, for example, be established by comparison with sensory evaluation data.

Table 3. Relative Concentrations of the Important Aroma Compounds of Black Currant Juice As Determined by GC-MS for Each of the Four Isolation Methods

		amount of compounds[b]			
no.[a]	compound	static headspace	P&T 15	P&T 60	solvent extraction
1	dimethyl sulfide	100 a	35 b	2 b	m [c]
2	methyl acetate	100 a	97 a	20 b	m
3	methyl 2-methylpropanoate	22 b	89 a	100 a	m
4	ethyl propaonate	7 c	35 b	100 a	m
5	2,3-butanedione	12 c	55 b	100 a	21 c
5	methyl butanoate	10 c	40 b	100 a	2 c
6	methyl 2-methylbutanoate	11 c	49 b	100 a	0 c
6	2-methylpropyl acetate	10 c	39 b	100 a	2 c
7	ethyl butanoate	9 c	36 b	100 a	1 c
8	ethyl 3-methylbutanoate	12 c	44 b	100 a	0 c
9	methyl-*trans*-2-butenoate	5 c	27 b	100 a	2 c
10	2- and 3-methylbutyl acetate	8 c	35 b	100 a	1 c
11	isocineole	6 c	30 b	100 a	1 c
12	cineole	6 c	30 b	100 a	2 c
13	mixture of 2- and 3-methyl-1-butanols	6 c	35 b	100 a	12 c
14	ethyl hexanoate	6 c	35 b	100 a	1 c
15	hexyl acetate	3 c	33 b	100 a	20 b
16	octanal	0 b	27 b	100 a	0 b
19	6-methyl-5-hepten-2-one	0 c	25 b	100 a	1 c
20	*cis*-rose oxide	0 c	22 b	100 a	0 c
21	methyl 2-hydroxybutyrate	3 d	21 c	100 a	58 b
21	*cis*-3-hexen-1-ol	3 c	23 b	100 a	15 b
22	methyl octanoate	0 c	32 b	100 a	0 c
23	nonanal	0 b	24 b	100 a	5 b
25	2-methoxy-3-isopropylpyrazine	0 c	27 b	100 a	10 bc
26	acetic acid	2 b	3 b	6 b	100 a
29	decanal	0 b	0 b	100 a	0 b
30	camphor	0 c	31 b	100 a	0 c
30	1,4-dimethyl-3-cyclohexenylmethyl ketone	0 c	25 b	100 a	0 c
31	3-methoxy-2-isobutylpyrazine	0 b	7 b	100 a	0 b
33	linalool	2 b	14 b	100 a	10 b
34	4-terpineol	2 c	17 b	100 a	17 b
35	butanoic acid	1 b	2 b	7 b	100 a
36	2- and 3-methylbutyric acid	0 b	0 b	0 b	100 a
40	α-terpineol	0 c	12 c	100 a	43 b
41	phellandral	0 b	0 b	100 a	0 b
46	β-damascenone	0 c	20 b	100 a	7 bc
49	benzyl alcohol	0 c	2 c	17 b	100 a
50	2-phenylethanol	0 c	2 c	13 b	100 a
55	4-methylphenol	2 d	22 c	100 a	46 b
57	eugenol	0 b	0 b	0 b	100 a
58	4-vinyl-2-methoxyphenol	0 b	0 b	0 b	100 a

[a] Numbers correspond to compounds in **Table 1**. Only compounds of high enough concentrations to be quantified are included. The highest concentration of each compound based on single ions is set to 100; the other concentrations corresponding to this value. [b] Letters a–d are used to compare mean values ($n = 3$) within each row, indicating significantly different results by Duncan's multiple-range test. [c] Detection started at RI 950 due to the solvent peak.

However, sensorial comparison of isolates of headspace methods with solvent extracts is not straightforward. Van Ruth et al. (*21*) investigated flavor release of rehydrated vegetables and found that dynamic headspace with mastication did not differ significantly from direct oral vapor release in the mouth, whereas purge and trap without mastication gave higher and dynamic headspace collection lower GC chromatogram peak areas.

The static headspace approach simulates the odor of the headspace in a food package as it is experienced by orthonasal perception. By purge and trap, on the other hand, some components are more enriched than others, and the composition does not reflect the gas phase at equilibrium, as perceived by the nose. Nevertheless, the detection limit of purge and trap is lower than that of static headspace. A solvent extract does to an even less extent reflect the sensory impression of a food, but it facilitates the identification of some low-volatility components. Depending on the purpose of the investigation, a solution could be to select both a method reflecting the sensory perception and a low detection limit method.

ABBREVIATIONS USED

NIF, nasal impact frequency; SNIF, surface of nasal impact frequency; GC-O, gas chromatography−olfactometry; GC-MS, gas chromatography−mass spectrometry; CHARM, combined hedonic aroma response measurement; AEDA, aroma extract dilution analysis; RI, retention index; FID, flame ionization detector; P&T, purge and trap; SD, standard deviation; LSD, least significant difference.

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Paper III

Recovery of volatile aroma compounds from black currant juice using vacuum membrane distillation

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Bagger-Jørgensen, R., Meyer, A. S., Varming, C. and Jonsson, G.

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Recovery of volatile aroma compounds from black currant juice by vacuum membrane distillation

Rico Bagger-Jørgensen [a], Anne S. Meyer [a,*], Camilla Varming [b], Gunnar Jonsson [c]

[a] *Food Biotechnology and Engineering Group, Technical University of Denmark, BioCentrum-DTU, Building 221, 2800 Lyngby, Denmark*
[b] *Department of Dairy and Food Science, The Royal Veterinary and Agricultural University, DK-1958 Frederiksberg C, Denmark*
[c] *Department of Chemical Engineering, Technical University of Denmark, Building 229, 2800 Lyngby, Denmark*

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Abstract

This study evaluated the recovery of seven characteristic black currant aroma compounds by vacuum membrane distillation (VMD) carried out at low temperatures (10–45 °C) and at varying feed flow rates (100–500 l/h) in a lab scale membrane distillation set up. VMD at feed flow from 100 to 500 l/h at 30 °C gave concentration factors, calculated for each aroma compound as $C_{permeate}/C_{feed}$, from ~4 to 15. The concentration factors increased with decreased juice temperature during VMD; at 10 °C concentration factors of 21–31 were obtained for the highly volatile aroma esters. The recovered levels of the highly volatile aroma compounds ranged from 68 to 83 vol.% with a feed volume reduction of 5 vol.% (10 °C, 400 l/h). The theoretically predicted aroma recovery as a function of the feed volume reduction was in accordance with the experimentally obtained values. VMD thus turned out to be a promising technique for gentle stripping of black currant juice aroma compounds.

Keywords: Vacuum membrane distillation; Black currant juice; Aroma recovery

1. Introduction

Aroma profiles of fruit juices usually comprise a mixture of a large number of volatile organic compounds. The individual aroma components differ according to their molecular structure, which in turn defines the solubility, the boiling point, and the volatility of each type of compound (Ramteke, Eipeson, & Patwardhan, 1990). In general, the aroma components are present in different concentrations and combinations, where the concentrations of individual aroma substances in common fruit juices usually range from less than 1 to 20 ppm (Śulc, 1984). The unique aroma profile of black currant (*Ribes nigrum* L.) juice comprise more than 60 constituents with a certain profile of terpenoids, aliphatic esters, carbonyl compounds and alcohols that make up the characteristic black currant aroma of the juice (Leino & Kallio, 1993). Since the aroma of black currant juice thus depends on the balanced presence of both poorly and highly volatile compounds, a minimization of thermal discrimination of volatiles during processing is desirable (Kollmansberger & Berger, 1994).

One of the basic unit operations of fruit juice technology is the concentration process where the solids content of the juice is increased from 10% to 12% up to 65–75% by weight (Śulc, 1984). The fruit juices are concentrated to reduce liquid volume, which in turn lowers storage, packaging and transport costs. An increased concentration of solids also assists in preventing microbial spoilage of the juice concentrate (Downes, 1990). In industrial juice processing plants, the juice concentration step is usually coupled with aroma-stripping and the stripped aroma concentrate is later added back to the concentrated juice (Śulc, 1984). Today, the predominantly used method for fruit juice concentration and aroma-stripping comprises one or several multistage falling film vacuum evaporators connected to a separate aroma recovery plant. The volatile aroma compounds are removed in the vapour phase obtained through falling film evaporation and subsequently trapped by condensation in an aroma recovery unit, where the efficiency of the trapping varies depending on the particular conditions and on the aroma compounds in question (Piggott, Paterson, & Clyne, 1993). The aroma

* Corresponding author. Fax: +45-45-49-88-22.
E-mail address: am@biocentrum.dtu.dk (A.S. Meyer).

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Nomenclature

C	concentration (kg/m^3)	M_i	molar mass (kg/mol)
J_v	volume flux (m/s)	N_i	mass flux (kg/s m^2)
k	mass transfer coefficient (m/s)	Δp_i	partial pressure gradient (Pa)
K	concentration factor (–)	T	temperature (°C)
K_m	permeability coefficient (–)	V	molar volume (m^3/mol)

compounds are thus subject to high temperature rectification (counter-current distillation), condensation and washing. During high temperature distillation the aroma profile of black currant juice has been demonstrated to undergo an irreversible change including formation of furan derivatives and sulphides, an increase in the concentration of aldehydes and a general decrease in the concentration of terpenoids (von Sydow & Karlsson, 1971a, 1971b). Thus, in addition to a significant consumption of energy, the conventional aroma stripping process coupled to the juice concentration has serious drawbacks, that include heat induced transformations of sensory attributes (colour, taste and aroma) and loss of nutrients (vitamin C) (Lazarides, Iakovidis, & Schwartzberg, 1990). Besides these major chemical and organoleptical changes, the overall aroma transfer with the conventional aroma recovery unit is imperfect, transferring only 40–65% of the total volatiles into the aroma concentrate (Śulc, 1991).

In recent years, novel membrane processes such as membrane distillation (MD), reverse osmosis (RO), and pervaporation have been evaluated as alternative membrane based separation and concentration processes in fruit juice and beverage technology (Calabrò, Jiao, & Drioli, 1994; Girard & Fukumoto, 2000; Laganà, Barbieri, & Drioli, 2000). Vacuum membrane distillation (VMD) is based upon using a microporous hydrophobic membrane for the separation of an aqueous feed solution into a retentate and a permeate by means of the pressure difference induced by the vacuum on the permeate side: the principle is that the liquid stream vaporizes at the membrane surface and the vapour diffuses through the gas phase inside the membrane pores (Mulder, 1996). The driving force of the process corresponds to the partial pressure gradient across the membrane. The conductive heat transfer across the membrane is negligible because of the low pressure on the permeate side (Lawson & Lloyd, 1996). The mass transfer through the membrane pores pre-dominantly take place according to the Knudsen mechanism implying that the different molecules move independently of each other (Mulder, 1996).

The aim of this study was to evaluate the potential of VMD to recover black currant juice aroma. This paper reports the influence of feed temperature and flow rate on the fluxes and concentration factors of seven characteristic black currant aroma compounds using a laboratory scale VMD set up. Furfural was selected as a compound to indicate heat treatment, whereas methyl butanoate, ethyl butanoate, ethyl hexanoate, *cis*-3-hexene-1-ol, 1,8 cineole and β-damascenone were selected because they are black currant impact compounds as determined by GC-sniffing of black currant berries and/or juice isolates (Iversen, Jakobsen, & Olsen, 1998; Latrasse, Rigaud, & Sarris, 1982). The components furthermore represent different chemical groups (Fig. 1).

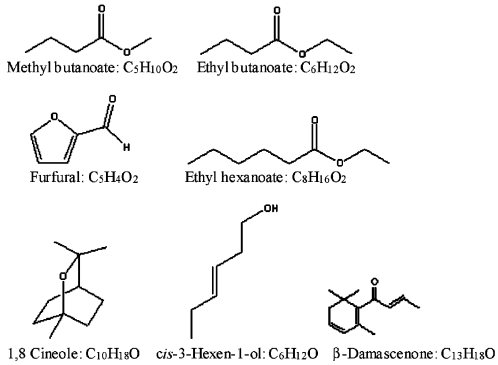

Fig. 1. Characteristic black currant aroma compounds selected for quantification in this study.

2. Materials and methods

2.1. Black currant juice

Juice produced from black currant (*Ribes nigrum*) cv. Ben Lomond was sampled directly after filtration, but prior to concentration, from an industrial juice process line (Vallø Saft A/S, Vallø, Denmark). The berries had been crushed, treated with enzymes (pre-press pectinases), and pressed. The juice had then been pasteurized, clarified conventionally with gelatin-silica sol, centrifuged, and ultrafiltered (cut off value 200,000 Dalton). The sugar, acid and turbidity levels of the juice were 12 °Brix, 33 g/kg (tartaric acid, pH 7.0) and <5 For-

mazan Nephelometric Units (FNU), respectively. The generally used criterion for clarity of the final black currant juice is a turbidity level less than 5 FNU at 3.0 °Brix as evaluated by nephelometry (A.I.J.N, 1996). The juice was kept frozen (−20 °C) in portions of 25 l until use. Prior to experimental VMD the juice samples were gently thawed at room temperature (20 °C) and heated to the experimental temperature.

2.2. Experimental set-up

The experimental set-up has been described previously by Izquierdo-Gil and Jonsson (2003). Based on the results obtained by Izquierdo-Gil and Jonsson (2003), a flat polytetrafluoroethylene (K150) membrane (surface area 37 cm^2) with support and a pore size of 0.1 μm was used (Osmonics, Minnetonka, USA). All data presented are averages of at least three measurements and are in tables and figures shown with their standard deviation. Data were compared using a 5% level of significance.

2.3. Dynamic headspace collection

Samples were thawed, and 50.0 g of juice or 5.0 g of aroma distillate were weighed into a 500 ml glass flask equipped with a purge head. Each sample was diluted with water to 150 g, and 1.00 ml of an internal standard (50 μl/l 4-methyl-1-pentanol, Aldrich, Steinheim, Germany) was added. The sample was placed in a waterbath at 30 °C and incubated for 10 min. Under magnetic stirring (200 rpm) samples were purged with nitrogen (200 ml/min) for 60 min. Volatiles were collected on Tenax-GR traps (250 mg) with mesh size 60/80 (Buchem bv, Apeldorn, Holland).

2.4. GC-MS analysis

The collected volatiles were thermally desorbed using an Automated Thermal Desorber (ATD 400, Perkin Elmer, USA). Desorption time from the trap to the cold trap was 15 min at 25 °C, with a helium flow of 60 ml/min. Volatiles were desorbed from the cold trap to the GC-column by flash heating from 5 to 300 °C. Separation and identification of aroma compounds was carried out on a Hewlett-Packard (Palo Alto, CA) G1800A System (GC-MS) equipped with a J & W Scientific DB-Wax column (30 m × 0.25 mm × 0.25 μm) using helium as the carrier gas (1 ml/min). The GC-MS split ratio was 1:10. The column temperature was kept at 40 °C for 10 min, increased by 6 °C/min to 240 °C, and kept isothermally for 25 min. The mass selective detector used the electron ionisation mode and an m/z scan between 10 and 425. Identifications were carried out by probability-based matching with mass spectra in the Wiley Library (Hewlett-Packard, Palo Alto, CA), and by comparisons with mass spectra and retention times of authentic reference compounds. Peak areas were calculated on the basis of total ion chromatograms and results are given as relative concentrations (peak area divided by internal standard area).

3. Results

3.1. Mass balances

The mass balances (MB) were calculated according to the formula below (1), i.e. for each individual aroma compound the sum of the recovered level in the final retentate and the collected permeate was divided by the level present in the initial feed, where C designates concentration and V designates volume:

$$\mathrm{MB} = \left[\frac{\left[(C \times V)_{\text{retentate}} + (C \times V)_{\text{permeate}} \right]}{(C \times V)_{\text{feed}}} \right] \times 100 \quad (1)$$

Except for furfural at 45 °C, that behaved differently than the other compounds (as discussed below), the total recovered amounts of each aroma compound were

Table 1
Mass balance: feed flow experiments[a]

Feed		Amount of aroma compound accounted for (%) shown with the standard deviation						
Flow (l/h)	Flux[b] (l/h/m^2)	Methyl butanoate	Ethyl butanoate	1,8 Cineole	Ethyl hexanoate	*cis*-3-Hexen-1-ol	Furfural	β-Damascenone
100	18.2[c]	106 ± 6	103 ± 3	95 ± 10	96 ± 1	101 ± 5	100 ± 4	101 ± 3
200	19.3[d]	102 ± 13	99 ± 11	87 ± 9	90 ± 12	99 ± 4	100 ± 3	84 ± 10
300	19.9[e]	108 ± 6	105 ± 6	101 ± 8	99 ± 4	106 ± 3	104 ± 1	94 ± 10
400	21.1[f]	90 ± 4	91 ± 6	97 ± 8	85 ± 11	101 ± 4	98 ± 6	93 ± 6
500	21.2[f]	97 ± 6[y]	96 ± 5[y]	106 ± 6[z]	97 ± 6[y]	107 ± 4[z]	109 ± 9[z]	92 ± 7[y]

Superscript letters c, d, e and f indicates grouping within a column with Duncan's multiple range test (Montgomery, 1991).
Superscript letters y and z indicates grouping within a row with Duncan's multiple range test (Montgomery, 1991).
No grouping indicates that there are not any significant differences within a row or column.
[a] Constant temperature: 30 °C.
[b] Flux through the membrane.

Table 2
Mass balance: temperature experiments[a]

Feed		Amount of aroma compound accounted for (%) shown with the standard deviation						
Temperature (°C)	Flux[b] (l/h/m^2)	Methyl butanoate	Ethyl butanoate	1,8 Cineole	Ethyl hexanoate	*cis*-3-Hexen-1-ol	Furfural	β-Damascenone
10	4.1^{c}	94 ± 7	96 ± 7	100 ± 5	98 ± 9	104 ± 3	101 ± 7^{c}	98 ± 5
15	5.7^{d}	100 ± 5	98 ± 5	97 ± 5	99 ± 3	106 ± 9	99 ± 9^{c}	96 ± 7
20	9.6^{e}	91 ± 7	97 ± 2	101 ± 8	96 ± 12	100 ± 3	104 ± 10^{c}	101 ± 8
25	15.2^{f}	96 ± 8	93 ± 4	101 ± 5	87 ± 12	99 ± 4	100 ± 3^{c}	94 ± 4
30	21.0^{g}	90 ± 4	91 ± 6	97 ± 8	85 ± 11	101 ± 4	98 ± 6^{c}	93 ± 6
35	27.7^{h}	94 ± 11	92 ± 3	94 ± 5	86 ± 3	96 ± 4	100 ± 2^{c}	84 ± 12
40	36.9^{i}	100 ± 8	97 ± 10	96 ± 8	97 ± 3	98 ± 7	105 ± 13^{c}	92 ± 5
45	48.0^{j}	95 ± 4^{x}	95 ± 9^{x}	91 ± 11^{x}	84 ± 6^{x}	105 ± 8^{y}	126 ± 12^{d,z}	85 ± 11^{x}

Superscript letters c, d, e, f, g, h, i and j indicates grouping within a column with Duncan's multiple range test (Montgomery, 1991).
Superscript letters x, y and z indicates grouping within a row with Duncan's multiple range test (Montgomery, 1991).
No grouping indicates that there aren't any significant differences within a row or column.
[a] Constant feed flow: 400 l/h.
[b] Flux through the membrane.

satisfactory as they ranged from 84% to 109% (Tables 1 and 2). At the experimental temperature of 45 °C the recovery of furfural was 126% (Table 2); this significant increase in the recovered level of furfural indicates that the temperature of 45 °C tended to facilitate furfural formation. There was no specific pattern in the overall variation in the recovered amounts, but the highest parameter settings resulted in significant variations in the mass balance levels accounted for. Presumably, these variations are a result of an unavoidably larger risk of aroma loss during sampling at the high feed flow rate (500 l/h) or at the high temperature (45 °C) than at the lower feed rates and temperatures (Tables 1 and 2).

The parameter settings significantly influenced the flux. A significant increase in flux was seen with increased temperature from 10 to 45 °C and with increased feed flow from 100 to 400 l/h, while a tendency of the flux to level off was observed when the feed flow was increased from 400 to 500 l/h (Tables 1 and 2). The measured permeate pressure was dependent on feed temperature, and increased from 7 mbar at 10 °C to 30 mbar at 45 °C (data not shown).

3.2. Concentration factors

The concentration factor (CF) of each aroma compound was calculated as the ratio between the concentration of the aroma compound in the permeate (C_{permeate}) and the corresponding initial feed concentration (C_{feed}).

$$\text{CF} = C_{\text{permeate}}/C_{\text{feed}}. \quad (2)$$

An increase in the feed flow resulted in a significant increase in the concentration factor of each compound (Table 3). At the highest flow rate of 500 l/h, at the constant feed temperature of 30 °C, the concentration factors ranged from 7 to 15.5, and were highest for the three esters methyl butanoate, ethyl butanoate, and ethyl hexanoate, and, in general, lowest for *cis*-3-hexen-1-ol and β-damascenone (Table 3). At each flow rate, the concentration factors of the aroma compounds could be categorized into significantly different groups according to their volatility with the highest concentration factors achieved for the highly volatile ester compounds: methyl butanoate, ethyl butanoate, and ethyl hexanoate (Table 3).

Table 3
Concentration factors: feed flow experiments[a] shown with the standard deviation

Flow (l/h)	Methyl butanoate	Ethyl butanoate	1,8 Cineole	Ethyl hexanoate	*cis*-3-Hexen-1-ol	Furfural	*β*-Damascenone
100	10.1 ± 0.8^{b,z}	9.8 ± 0.3^{b,z}	10.0 ± 1.2^{b,z}	9.0 ± 0.5^{b,y}	5.2 ± 0.3^{b,x}	5.8 ± 0.3^{b,x}	3.8 ± 0.1^{b,v}
200	12.6 ± 0.3^{c,z}	12.2 ± 0.6^{c,zy}	9.5 ± 0.2^{b,x}	11.8 ± 0.3^{c,y}	4.8 ± 0.3^{b,vu}	5.4 ± 1.3^{b,v}	4.5 ± 0.5^{b,u}
300	13.0 ± 0.8^{c,z}	12.1 ± 1.0^{c,zy}	9.9 ± 1.3^{b,x}	11.5 ± 1.2^{c,y}	5.6 ± 0.3^{bc,v}	6.0 ± 0.6^{b,v}	5.4 ± 0.2^{c,v}
400	12.7 ± 1.7^{c,zy}	13.1 ± 0.2^{c,zy}	12.2 ± 0.4^{c,y}	13.7 ± 0.4^{d,z}	6.1 ± 0.7^{c,v}	5.9 ± 0.7^{b,v}	7.5 ± 1.2^{d,x}
500	15.4 ± 1.9^{d,z}	15.2 ± 1.8^{d,z}	14.3 ± 2.1^{d,z}	15.5 ± 2.1^{e,z}	7.1 ± 0.8^{d,y}	8.9 ± 1.7^{c,y}	9.0 ± 0.8^{c,y}

Superscript letters b, c, d and e indicates grouping within a column with Duncan's multiple range test (Montgomery, 1991).
Superscript letters u, v, x, y and z indicates grouping within a row with Duncan's multiple range test (Montgomery, 1991).
[a] Constant temperature: 30 °C.

Table 4
Concentration factors: temperature experiments[a] shown with the standard deviation

Temperature (°C)	Methyl butanoate	Ethyl butanoate	1,8 Cineole	Ethyl hexanoate	*cis*-3-Hexen-1-ol	Furfural	*β*-Damascenone
10	$21.1 \pm 2.9^{b,x}$	$24.6 \pm 2.5^{b,y}$	$20.2 \pm 0.7^{b,x}$	$31.4 \pm 3.0^{b,z}$	$9.3 \pm 0.5^{b,u}$	$8.9 \pm 0.7^{b,u}$	$11.5 \pm 1.0^{b,v}$
15	$17.1 \pm 1.3^{c,v}$	$20.9 \pm 0.8^{c,y}$	$18.6 \pm 1.2^{c,x}$	$23.6 \pm 0.9^{c,z}$	$9.3 \pm 0.2^{b,ut}$	$8.5 \pm 1.0^{b,t}$	$10.2 \pm 1.4^{b,u}$
20	$16.1 \pm 0.3^{c,x}$	$18.3 \pm 0.2^{d,y}$	$16.3 \pm 1.5^{d,x}$	$19.9 \pm 2.1^{d,z}$	$6.0 \pm 0.7^{d,u}$	$5.2 \pm 0.5^{c,u}$	$8.3 \pm 0.8^{c,v}$
25	$14.5 \pm 1.4^{d,zy}$	$14.6 \pm 1.4^{e,zy}$	$13.4 \pm 0.9^{e,y}$	$15.0 \pm 1.6^{e,z}$	$6.7 \pm 0.3^{cd,v}$	$5.0 \pm 0.7^{c,u}$	$8.1 \pm 1.1^{c3,x}$
30	$12.7 \pm 1.7^{e,zy}$	$13.1 \pm 0.2^{f,zy}$	$12.2 \pm 0.4^{f,y}$	$13.7 \pm 0.4^{e,z}$	$6.1 \pm 0.7^{d,v}$	$5.9 \pm 0.7^{c,v}$	$7.5 \pm 1.2^{c,x}$
35	$10.6 \pm 1.3^{f,z}$	$10.9 \pm 1.2^{g,z}$	$11.1 \pm 0.7^{f,z}$	$11.1 \pm 1.6^{f,z}$	$7.1 \pm 1.3^{c,y}$	$5.6 \pm 1.0^{c,x}$	$7.3 \pm 1.8^{cd,y}$
40	$9.9 \pm 1.7^{f,z}$	$10.6 \pm 1.6^{g,z}$	$9.2 \pm 1.3^{g,z}$	$9.1 \pm 2.0^{g,z}$	$6.3 \pm 0.1^{cd,y}$	$5.3 \pm 0.6^{c,y}$	$6.9 \pm 1.4^{cd,y}$
45	$9.9 \pm 1.9^{f,z}$	$9.8 \pm 1.9^{g,z}$	$9.3 \pm 1.3^{g,z}$	$9.4 \pm 1.1^{g,z}$	$6.6 \pm 1.1^{cd,y}$	$5.2 \pm 1.0^{c,y}$	$5.9 \pm 1.6^{d,y}$

Superscript letters b, c, d, e, f and g indicates grouping within a column with Duncan's multiple range test (Montgomery, 1991).
Superscript letters t, u, v, x, y and z indicates grouping within a row with Duncan's multiple range test (Montgomery, 1991).
[a] Constant feed flow: 400 l/h.

Increased VMD temperature resulted in significantly decreased concentration factors for each compound (Table 4). The highest concentration factors of ~20–31 were thus obtained for the three esters and 1,8 cineole at 10 °C at a constant feed flow of 400 l/h (Table 4). At the highest experimental temperature of 45 °C, and at a constant feed flow of 400 l/h, the concentration factors for the different compounds were much lower and varied from 5 to 10, again with the highest values for the esters and 1,8 cineole. At the low temperature range the different aroma compounds' concentration factors generally differed significantly from each other. At the increased temperatures the difference diminished resulting in only two significantly different groups at 45 °C (Table 4). These differences confirmed that the esters and 1,8 cineole had higher concentration factors than the other compounds and that the differences were more pronounced at low temperature.

3.3. Volumetric amounts recovered

The amounts of recovered aroma at a 2% feed volume reduction varied between 15–50% and 13–23% at the temperatures 10 and 45 °C, respectively, at constant feed flow (Table 5). The extent of recovery differed among the seven compounds; with the esters and 1,8 cineole showing the highest recovery levels at both temperatures. The amounts of aroma recovered at 10 °C and at 45 °C differed significantly for all the aroma compounds except for furfural and *cis*-3-hexen-1-ol (Table 5).

Table 5
Recovered aroma (%) at 2% volume reduction shown with the standard deviation

Aroma compound	10 °C[a]	45 °C[a]
Methyl butanoate	36.5 ± 2.3^{z}	23.0 ± 1.9^{y}
Ethyl butanoate	40.2 ± 1.5^{z}	22.5 ± 2.4^{y}
1,8 Cineole	34.5 ± 1.9^{z}	20.6 ± 3.5^{y}
Ethyl hexanoate	50.2 ± 1.8^{z}	18.9 ± 3.1^{y}
cis-3-Hexen-1-ol	15.0 ± 2.0^{z}	13.6 ± 1.1^{z}
Furfural	16.2 ± 2.8^{z}	12.6 ± 1.6^{z}
β-Damascenone	19.3 ± 2.6^{z}	12.7 ± 2.4^{y}

Superscript letters y and z indicates grouping within a row with Duncan's multiple range test (Montgomery, 1991).
[a] Constant feed flow: 400 l/h.

When the amount of recovered black currant juice aroma was extended to constitute 5% of the total feed volume, the amounts of recovered aroma at 10 °C varied markedly among the selected aroma compounds (Fig. 2, exp. data). With a 5% volume reduction during VMD, the levels of recovered aroma were from 32% to 83%, with the highest levels of recovered aroma being obtained for ethyl hexanoate (83%), ethyl butanoate (74%), and 1,8 cineole (68%).

3.4. Membrane permeability

The water vapour pressure, and hence the transport of water through the membrane, depends on the temperature at the membrane surface. The relationship between the flux and the water vapour pressure difference

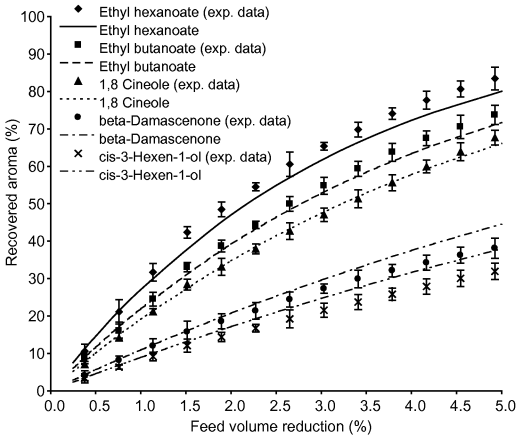

Fig. 2. The fractions of aroma recovered shown with the standard deviation (10 °C, 400 l/h) versus the theoretically calculated values of aroma recoveries (Eq. (5), the concentration factors (K) are from Table 4 (10 °C)).

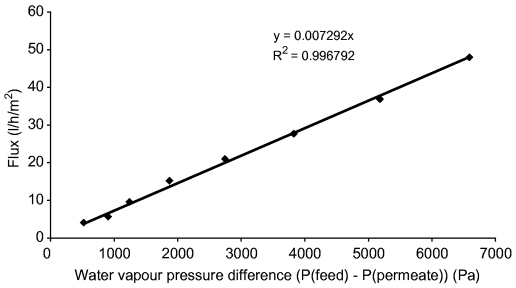

Fig. 3. The permeability of the membrane depicted as flux (of juice) vs. the water vapour pressure difference between the feed and permeate side of the membrane ($P_{feed} - P_{permeate}$).

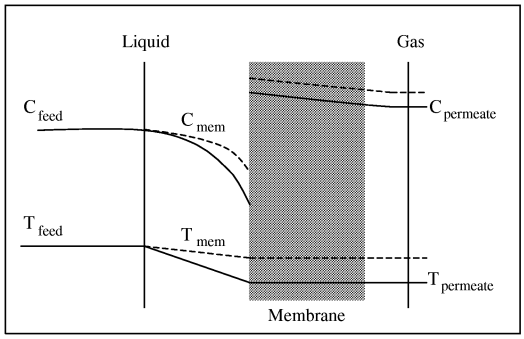

Fig. 4. Theoretically assumed profiles of concentration and temperature (°C) gradients in VMD (Couffin, Cabassud, & Lahoussine-Turcaud, 1998). The solid lines illustrate the profiles at low feed flow rate, while the dotted lines illustrate the profiles at high feed flow rate (not to scale).

of the feed and permeate is shown in Fig. 3. The correlation between the permeate flux and the water vapour pressure difference (P_{feed}-$P_{permeate}$) was practically linear in the range of operative conditions with $y = 0.007292x$ and $R^2 = 0.997$. This linear relationship signified, as expected, that the difference in water vapour pressure was the real driving force across the membrane. At the experimental conditions used (30 °C, 400 l/h) the influence of the temperature polarization was insignificant (Table 1) and the temperature polarization was therefore neglected in the above correlation.

4. Discussion

The feed flow was varied from 100 to 500 l/h, corresponding to Reynolds numbers in the region from 2000 to 8000 approximately, which is mainly in the turbulent region. Each increase in the experimental feed flow rate resulted in a significant increase in the permeate concentration and in the overall flux (the only exception was the increase in feed flow from 400 to 500 l/h that did not affect the flux). The influence of the feed flow rate on the permeate concentration was much larger than the influence on the overall flux. Thus, increasing the feed flow from 100 to 500 l/h resulted in an increase of minimum 36% (*cis*-3-hexen-1-ol) in the concentration factors of the aroma compounds, but only in an increased overall flux of 16% (calculated from data shown in Tables 1 and 3). With no further increase in the transmembrane flux an increase in the concentration factors was seen when the flow rate was raised from 400 to 500 l/h (Tables 1 and 3).

Fig. 4 illustrates the temperature and concentration profiles in VMD. During aroma stripping the temperature at the membrane surface will decrease because of the evaporation at the vapour–liquid interface (solid line: T_{mem}, Fig. 4). The evaporation fluxes achieved in VMD involve significant heat fluxes from the liquid bulk to the vapour–liquid interface; heat transfer across the boundary layers is therefore often the rate-limiting step for mass transfer, a phenomenon known as temperature polarization (Lawson & Lloyd, 1996). During aroma stripping the vapour–liquid equilibrium conditions at the membrane-feed boundary will differ from the bulk condition. Because of the large enrichment factor for the membrane process and the low initial aroma concentration (ppm) in the feed, the aroma concentration at the membrane surface will invariably decrease, a phenomenon known as concentration polarization (solid line: C_{mem}, Fig. 4).

An increase in the feed flow reduces temperature polarization, which consequently increases the temperature at the vapour–liquid interface (T_{mem}) (from the solid to the dotted line, Fig. 4), which in turn results in a higher overall flux. An increase in the feed flow also decreases the concentration polarization that in turn increases the concentration at the membrane surface (C_{mem}) (from the solid to the dotted line, Fig. 4), which results in a higher permeate concentration ($C_{permeate}$) (from the solid to the dotted line, Fig. 4). The influence of concentration polarization on VMD can be described in a way similar to RO as shown in Eq. (3) (Jonsson & Boesen, 1984), where J_v is the volume flux and k is the mass transfer coefficient.

$$\frac{C_{permeate} - C_{membrane}}{C_{permeate} - C_{feed}} = \exp\left(\frac{J_v}{k}\right) \quad (3)$$

An increase in the ratio (J_v/k) increases the concentration polarization and thereby the concentration at the membrane surface ($C_{membrane}$) decreases.

The results of the present study demonstrate that an increase in the feed flow rate decrease the heat and mass-transfer resistances in the liquid phase resulting in higher temperature and aroma concentration at the membrane surface and consequently a higher permeate flux and

concentration. However, due to the very high concentration factors of the aroma compounds the concentration polarization depends to a greater extent on the feed flow rate than the temperature polarization. This explains why the overall flux, in contrast to the permeate concentration, did not increase when the flow rate was raised from 400 to 500 l/h. A flow above 400 l/h will not significantly decrease the temperature polarization, whereas the limit for the concentration polarization has not yet been reached. For all seven aroma compounds the maximum concentration factors were obtained at 500 l/h (Table 3). Higher concentration factors than the ones obtained in this study should therefore be possible, since the limit where an increase in the feed flow does not significantly decrease the concentration polarization has not yet been reached. The results above are in accordance to what Urtiaga, Gorri, Ruiz, and Ortiz (2001) and Bandini and Sarti (1999) have reported for VMD with other volatile organic compounds.

Increasing the temperature significantly influenced the overall flux, which increased almost exponentially, a behaviour typically observed in MD processes (Bandini, Saavedra, & Sarti, 1997). The overall flux at 45 °C was 12 times larger than at 10 °C. It is evident from Eq. (3) that a higher flux (J_v) increases the concentration polarization. An increase of the temperature will increase the diffusivity of the aroma compounds in the feed solution; however, this will have only minor influence on the concentration polarization in comparison to the much higher permeate flux as expressed by the ratio (J_v/k). For this reason, the increases in temperature resulted in higher concentration polarization and thus lower concentration factors. The data obtained were consistent with this explanation for all seven aroma compounds since the maximum concentration factors achieved were obtained at the lowest examined temperature: 10 °C (Table 4). Higher concentration factors should therefore be possible since the permeate concentration increases with decreasing temperature. An experimental temperature less than 10 °C would, however, result in a transmembrane flux less than 4 l/h/m^2. With other volatile compounds Bandini et al. (1997) have obtained similar results using permeate concentrations of up to 25 times that of the feed.

From the differential mass balance, Eq. (4), the fraction of recovered aroma as a function of the feed volume reduction can be calculated by Eq. (5), where K is the concentration factor.

$$\mathrm{d}(V \times C) = C_{\mathrm{permeate}} \times \mathrm{d}V \tag{4}$$

$$\frac{V_{\mathrm{retentate}} \times C_{\mathrm{retentate}}}{V_{\mathrm{feed}} \times C_{\mathrm{feed}}} = \left(\frac{V_{\mathrm{retentate}}}{V_{\mathrm{feed}}}\right)^{K} \quad \text{with } K = \frac{C_{\mathrm{permeate}}}{C_{\mathrm{feed}}} \tag{5}$$

In Fig. 2 the theoretically recovered aroma fraction $(1 - (V_{\mathrm{retentate}}/V_{\mathrm{feed}})^K)$ as a function of the feed volume reduction $(1 - (V_{\mathrm{retentate}}/V_{\mathrm{feed}}))$ is compared with the experimental data. It is evident that the experimental and predicted values of the highly volatile compounds are in accordance. The concentration factor is not constant but concentration dependent, which might be the reason why the recovered amounts of the poorly volatile aroma compounds have a tendency to be less than the predicted values.

Table 5 shows the differences in aroma recovery between the temperatures 10 and 45 °C for the seven aroma compounds. At 10 °C the values of the aroma recovery are generally significantly larger than those obtained at 45 °C. In addition, the differences in volatility among the compounds are clearly reflected in the table where the highly volatile compounds exhibit a significantly higher degree of recovery. Within a 2% feed volume reduction the low degrees of recovery of furfural and *cis*-3-hexen-1-ol do not increase significantly with the temperature illustrating the difficulty in recovering the poorly volatile compounds. The ideal experimental temperature profile for VMD would be to begin with a low temperature for aroma recovery. Then, when a satisfactory level of aroma recovery has been achieved, the temperature and consequently the flux should be increased for water removal. It is evident from Eq. (3) that a decrease in temperature has a larger effect on the concentration factor than an increase in the feed flow rate. The volume flux (J_v), which is temperature dependent, increased 12 times whereas the mass transfer coefficient (k), which is dependent on the feed flow rate raised to the power 0.75 ($u^{0.75}$) (Jonsson & Boesen, 1984), increased only 3.3 times ($500^{0.75}/100^{0.75} \approx 3.3$).

The downstream pressure increased 23 mbar when the temperature was increased from 10 to 45 °C. Couffin et al. (1998) have reported that an increase in downstream pressure improved the selectivity of the process. The above results indicate that the separation rate of the seven aroma compounds was not influenced by the permeate pressure in the range of the experimental conditions: $7 < P < 30$ mbar.

The membrane used in the VMD experiments had a pore size of 0.1 μm. The mean free path of the diffusing species is usually larger than the pore size of the membrane and under such conditions; the Knudsen diffusion mechanism usually dominates the mass transfer through the membrane. So, the mass flux of the component i, N_i, is linearly related to its partial pressure gradient (Δp_i) across the membrane by the hydraulic permeability $K_m\sqrt{M_i}$ as follows (Iversen, Bhatia, Dam-Johansen, & Jonsson, 1997; Izquierdo-Gil & Jonsson, 2003).

$$N_i = K_m \times \sqrt{M_i} \times \Delta p_i \tag{6}$$

where M_i is the molar mass of i. K_m is the permeability coefficient and depends only on membrane geometry and structure (which are often difficult to obtain) as well as on temperature. It does not depend on the nature of

the gas going through the membrane. The linear correlation between the permeate flux and the vapour pressure difference presented in Fig. 3 shows that the Knudsen diffusion mechanism applies in the performed experiments with the hydraulic permeability ($K_m\sqrt{M_{water}}$) equal to 0.007292 kg/(h m^2 Pa). The permeability coefficient (K_m) was equal to 1.509×10^{-5} (s mol$^{1/2}$/m kg$^{1/2}$), which is 23% lower than the value determined on an identical type of membrane by Izquierdo-Gil and Jonsson (2003). A reason for this inconsistency could be that the water vapour pressure P (feed) in Fig. 3 was determined for pure water and not juice (containing 12% sugar). Pure water has a higher water vapour pressure than juice at a given temperature, which results in a lower hydraulic permeability and hence a lower permeability coefficient.

4.1. Comparison of VMD with the conventional method

The amount of vapour to be extracted for aroma separation using the conventional process is 10–45% of feed volume (Bielig & Gründing, 1985). Besides extracting a large amount of vapour for aroma separation the conventional aroma recovery plant transfers only 40–65% of the total volatiles into the aroma concentrate (Śulc, 1991). In VMD the amount of volatiles in the aroma permeate are substantially higher and the amount of vapour to be extracted is therefore considerably less as indicated in Fig. 2. When 5% of feed volume had been extracted, between 68% and 83% of the highly volatile compounds and between 32% and 38% of the poorly volatile compounds, had been recovered.

The complexity of fruit aroma volatiles makes it rather difficult to separate aroma volatiles from different fruit juices using one and the same aroma recovery plant. Today, there is no universal aroma recovery plant with which highly and poorly volatile aroma compounds of every fruit type can be separated, rectified and concentrated with the same efficiency (Ramteke et al., 1990). VMD may be a suitable technique for satisfactory conservation of the original qualities of thermo sensitive aroma compounds in highly aromatic fruit juices like black currant juice. Vacuum conditions can shift the equilibria present between vapour phase and liquid phase at atmospheric pressure making azeotropic recovery possible (Bielig & Gründing, 1985; Ramteke, Singh, Rekha, & Eipeson, 1993).

5. Conclusions

The positive, practically linear correlation between the permeate flux and the water vapour pressure difference across the membrane signified that the Knudsen diffusion mechanism applied in the performed experiments and that the difference in water vapour pressure was the driving force of the VMD process.

When the heat and mass-transfer resistances in the liquid phase decrease, the results are a higher temperature and a higher aroma concentration at the membrane surface and consequently higher permeate flux and concentration. The experimental data also showed that high feed flow rate and low temperature gave the highest concentration factors. The overall flux decreased almost exponentially with decreased temperature and a change in temperature had a larger effect on the concentration factors than a change in the feed flow rate. The highest concentration factors achieved in this study were 21–31 (10 °C, 400 l/h) and were obtained for the most volatile black currant aroma esters. At 5 vol.% feed volume reduction the amount of recovered aroma was between 68 and 83 vol.% of the highly volatile compounds and between 32 and 38 vol.% of the poorly volatile compounds.

From a differential mass balance over the process, the theoretically predicted aroma recoveries were calculated as a function of the feed volume reduction at 10 °C using the experimentally determined concentration factors. The experimental and the predicted values of especially the highly volatile compounds were in very good accordance.

The data suggest that VMD is a promising technique for gentle aroma stripping of thermally sensitive fruit aroma compounds. VMD is, however, only one of several known MD techniques. Other MD techniques include sweeping gas MD, osmotic MD and direct contact MD. The applicability of these techniques for aroma stripping deserves further investigation.

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Paper IV

Influence of thermal treatment on black currant juice (*Ribes nigrum* L.) aroma

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Influence of Thermal Treatment on Black Currant (*Ribes nigrum* L.) Juice Aroma

CAMILLA VARMING, MOGENS L. ANDERSEN, AND LEIF POLL*

Department of Food Science, Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

The influence of thermal treatment on black currant juice aroma was investigated in temperature and time ranges relevant for black currant juice concentration processes, namely, 45, 60, 75, and 90 °C. Forty-nine aroma compounds were quantified, and the thermal treatment resulted in concentration increases of most terpenes, aldehydes, furans, and phenols, whereas the concentration of esters slightly decreased. Higher temperatures and longer exposure times had larger effects on the aroma compounds. Odor triangle tests showed no sensory difference between pasteurized juice and juice heated at 60 °C, whereas juice heated at 90 °C differed significantly from pasteurized juice. It is concluded that a 90 °C thermal treatment of black currant juice, which is in the temperature range used for conventional evaporation of black currant juice, has an effect on the aroma and sensory properties.

KEYWORDS: Aroma; black currant juice; thermal treatment; dynamic headspace collection

INTRODUCTION

Black currants are almost exclusively grown in Europe (*1*) and are mainly processed as juice, syrup, or jam (*2*). The processing of the berries usually requires the application of heat, which causes changes in the aroma and sensory characteristics of the fruit. The industrial production of black currant berries to juice involves enzyme treatment, pressing, clarification, and filtration as well as several heating steps. Each processing step alters to some extent the aroma profile, mainly by a decrease in the concentration of aroma components (*3*). More than 120 aroma compounds have been identified in black currant juice, in which terpenes, esters, and alcohols are the main groups of aroma compounds (*4*−*7*).

von Sydow and co-workers studied the influence of heat on the aroma of black currant products and found that heating black currant juice or mash to 70, 85, or 100 °C for 30 min increased the levels of benzene derivatives, dimethyl sulfide, and aldehydes and caused large rearrangements in the monoterpene complex. Larger changes in the composition of the volatiles were observed at higher temperatures than at lower (*8*, *9*). Heating of black currants leads to an increase of odor qualities such as cooked odor and a decrease of odors such as fruity and floral (*10*).

Short-time heating has been shown to cause minor or no aroma and sensory changes. Heating of black currant juice to 80 °C for 4 min did not lead to significant changes in the content of volatile compounds (*4*). Pasteurization of black currant nectar at 88 °C for 27 s caused only minor changes in the concentration levels of 2 of 52 compounds, and no significant differences between pasteurized and nonpasteurized nectar were found in a sensory evaluation (*5*).

Fruit juices are often concentrated to reduce volume and stabilize the product for storage and transport. The conventional method of concentrating fruit juices is by evaporation of water and recovery of the aroma in a distillate, which is subsequently added to the concentrate for reconstitution. During falling film evaporation concentration processes temperatures of 90−105 °C are applied with a residence time of ∼5−10 min (*11*, *12*). The aroma of concentrated juice is distinctly different from that of fresh black currant juice. An overall loss of some esters, alcohols, carbonyls, and terpenes occurs during the concentration, whereas certain volatiles are recovered in larger amounts than in the base juice. This is the case for furan and benzene derivatives, indicative of heat treatment, and aliphatic and terpene alcohols that might have existed in glycosidically or otherwise bound forms (*6*, *7*). Partial least-squares regression has been successful in modeling the perceived sensory flavor intensity of black currant drinks from aroma data on concentrate composition (*13*).

Recently, gentler membrane process technologies for distillation and concentration of fruit juices have been introduced (*14*). One such process is vacuum membrane distillation, which require less or no heat admission as the process is conducted in the 10−60 °C range (*15*, *16*).

The aim of the present study was to investigate thermally induced changes in the composition of aroma compounds from black currant juice by using temperatures and holding times that are relevant for conventional and novel black currant juice concentration processes. Several temperature and holding time levels have been investigated, and a large range of aroma compounds are quantified, with emphasis on characterization of the changes in the concentration of compounds that are important for the aroma of black currants.

MATERIALS AND METHODS

Materials. A commercial black currant juice of the variety Ben Lemond was obtained from an industrial plant. The juice preparation included crushing, heating, enzyme treatment (50 °C/maximum 6 h), pressing, pasteurization (98 °C/30 s), clarification (45 °C/maximum 6 h), and filtration. The juice was stored at −18 °C and defrosted

* Author to whom correspondence should be addressed [telephone (+45)-35283240; fax (+45)35283265; e-mail lep@kvl.dk].

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overnight at 5 °C before use. °Brix of the juice was 12, and the pH was 3.0. The aroma standards used for quantification were obtained as follows: ethyl propanoate, methyl butanoate, ethyl butanoate, ethyl 3-methylbutanoate, butyl acetate, methyl hexanoate, ethyl hexanoate, eugenol, furfural, ethyl benzoate, and benzyl alcohol (Merck, Darmstadt, Germany); α-terpipene, rose oxide, linalool oxide, camphor, cumin aldehyde, methyl 2-furoate, and *p*-cymene (Fluka, Buchs, Switzerland); 4-methylphenol (Supelco, Bellefonte, PA); terpinolene and 4-terpineol (Roth, Karlsruhe, Germany); hexyl acetate (K&K Laboratories, Plainview, NY); β-damascenone (Firmenich, La Plaine, Switzerland); benzaldehyde (Riedel-de Häen, Seelze, Germany); and the remaining compounds were from Sigma-Aldrich (Copenhagen, Denmark).

Heating of Juice. Two hundred grams of black currant juice was weighed into a 500 mL blue-cap flask equipped with a screw cap. Heating was performed in a closed system to avoid evaporation. To obtain the desired temperatures of 45, 60, 75, and 90 °C, samples were heated in a microwave oven (Samsung Classic Collection microwave oven-b30) for 57, 80, 110, and 130 s, respectively. To facilitate heat distribution within samples, each sample was shaken halfway through the heating time. Samples were then transferred to a preheated water bath and, under magnetic stirring, kept at the desired temperatures for 2.5, 5, 10, 30, and 60 min, respectively. One sample, labeled "0", was not further heated after heating in the microwave oven. The control sample, henceforth termed the pasteurized juice, was not subject to any heating, apart from the juice preparation described under Materials.

Immediately after heating at the desired temperature, samples were cooled in an ice−water bath and stored at 5 °C overnight. For aroma analyses each time and temperature combination was performed in triplicate. For sensory analyses the juice was heated prior to each of four triangle test sessions.

Dynamic Headspace Collection. Seventy-five grams of black currant juice was weighed into a 250 mL glass flask equipped with a purge head. One milliliter of internal standard (50 μL/L 4-methyl-1-pentanol, Aldrich, Steinheim, Germany) was added. Sample temperature was equilibrated in a 30 °C water bath for 10 min. Under magnetic stirring (200 rpm) the sample was then purged with nitrogen (100 mL/min) for 45 min. The volatiles were collected into traps containing 250 mg of Tenax TA (mesh size = 60/80, Buchem bv, Apeldoorn, The Netherlands).

Quantification. Quantifications for dynamic headspace collection were carried out on commercially available aroma compounds constituting >0.1% of the total peak area of the black currant juice GC chromatogram. A tap water model solution resembling black currant juice (glucose concentration = 48 g/L, fructose concentration = 61 g/L, citric acid monohydrate concentration = 39 g/L, 14 °Brix) was used. The aroma compounds to be quantified were divided into two series. For each series, 100 mg/L of aroma standard stock solutions in ethanol was diluted with the model solution to concentrations of 0.1 and 0.5 mg/L. During data processing linear calibration curves of each aroma standard were based on entries of 0, 0.1, and 0.5 mg/L. Due to their high concentrations in the juice, methyl butanoate and 2-methyl-1-propanol were analyzed at an additional concentration level of 1.0 mg/L, and 2-methyl-1-butanol was analyzed also at 5.0 mg/L. Dynamic headspace collection was performed in triplicate, under the same conditions as applied to the juice samples.

Gas Chromatography−Mass Spectrometry (GC-MS). The collected volatiles from the juice and the model solution were thermally desorbed using an automated thermal desorber (ATD 400, Perkin-Elmer), and separation and identification of aroma compounds were carried out on a Hewlett-Packard (Palo Alto, CA) G1800A S GC-MS system equipped with a J&W Scientific DB-Wax column (30 m × 0.25 mm × 0.25 μm). Settings were the same as described in ref *17*. Identifications were carried out by probability-based matching with mass spectra in the G1035A Wiley library (Hewlett-Packard) and comparisons with mass spectra and retention indices (RI) of the authentic reference standards. Linear retention indices were calculated after analysis under the same conditions of an *n*-alkane series (C9−C24). Peak area calculations were based on single ions, and peak areas of aroma compounds were divided by peak area of the internal standard.

Principal Component Analysis (PCA). To study the main variation in the material, multivariate data analysis was applied. In principal component analyses average response values over aroma replicates were used, and variables were mean centered and scaled to unit variance prior to analysis. The calibration models were validated by full cross validation and results presented as validation variation. Analyses were performed using the Unscrambler (Windows version 7.6 software package, Camo a/s Trondheim, Norway).

Sensory Evaluation. Triangle tests were used to evaluate odor differences between the heat-treated and the original pasteurized black currant juice samples. Twenty-eight untrained judges were selected from staff and students of the department. The thermal treatments to be tested were selected on the basis of preliminary test sessions and on temperature relevance for the juice concentration processes. Juice

Table 1. Concentration of Aroma Compounds in Pasteurized and Heated Black Currant Juice

compound	retention index	concn[a] (μg/L) pasteurized juice	concn[a] (μg/L) juice heated at 90 °C for 60 min
alcohols			
2-methyl-1-propanol	1078	618 ± 31	477 ± 97
2- and 3-methyl-1-butanol[b]	1200	3404 ± 78	2992 ± 161
1-hexanol	1344	72 ± 0.87	61 ± 3.5
cis-3-hexenol	1371	36 ± 0.61	32 ± 1.4
trans-2-hexen-1-ol	1392	26 ± 0.79	26 ± 1.9
1-octanol	1545	2.9 ± 0.29	3.3 ± 0.29
carbonyls			
trans-2-methyl-2-butenal	1069	6.4 ± 0.03	12 ± 1.3
octanal	1273	0.30 ± 0.14	1.7 ± 0.97
nonanal	1375	2.5 ± 0.44	16 ± 5.0
(*E*)-3-penten-2-one	1108	5.5 ± 0.20	48 ± 4.9
6-methyl-5-hepten-2-one	1320	0.63 ± 0.02	0.70 ± 0.05
esters			
methyl 2-methylpropanoate	910	1.7 ± 0.01	1.4 ± 0.07
ethyl propanoate	939	3.8 ± 0.04	3.0 ± 0.30
methyl butanoate	976	1007 ± 11	1053 ± 75
methyl 2-methylbutanoate	1000	1.1 ± 0.03	0.86 ± 0.11
2-methylpropyl acetate	1002	2.2 ± 0.04	1.5 ± 0.20
ethyl butanoate	1023	222 ± 2.4	194 ± 21
ethyl 3-methylbutanoate	1049	0.17 ± 0.004	0.14 ± 0.02
butyl acetate	1052	2.0 ± 0.05	1.5 ± 0.14
2-methylbutyl acetate	1111	2.2 ± 0.04	1.5 ± 0.20
methyl hexanoate	1176	17 ± 0.22	12 ± 1.5
ethyl hexanoate	1223	4.5 ± 0.01	3.0 ± 0.55
hexyl acetate	1261	0.48 ± 0.01	0.38 ± 0.10
methyl octanoate	1374	0.56 ± 0.01	0.35 ± 0.05
terpenoids			
α-terpipene	1165	3.1 ± 0.67	33 ± 8.0
limonene	1190	0.61 ± 0.15	5.1 ± 0.71
γ-terpinene	1229	0.84 ± 0.16	15 ± 3.4
p-cymene	1251	1.6 ± 0.04	9.1 ± 2.0
terpinolene	1265	0.35 ± 0.06	8.4 ± 1.9
rose oxide	1339	0.47 ± 0.01	0.47 ± 0.06
cis-linalool oxide	1425	22 ± 0.53	64 ± 6.3
camphor	1490	0.18 ± 0.02	0.48 ± 0.05
linalool	1530	18 ± 0.62	8.7 ± 0.17
4-terpineol	1585	385 ± 9.1	300 ± 6.8
β-cyclocitral	1599	4.3 ± 0.06	10 ± 1.4
α-terpineol	1679	65 ± 2.0	124 ± 7.1
cumin aldehyde	1760	0.0 ± 0.0	1.4 ± 0.19
phenols			
4-methylphenol	2059	0.0 ± 0.0	11 ± 1.9
eugenol	2146	2.8 ± 0.12	13 ± 2.1
carvacrol	2189	1.6 ± 0.32	4.8 ± 0.89
furans			
furfural	1439	6.0 ± 0.35	1077 ± 147
2-acetylfuran	1479	11 ± 1.3	107 ± 8.4
methyl 2-furoate	1553	3.3 ± 0.09	24 ± 3.4
others			
2-methoxy-3-isopropyl-pyrazine	1413	0.05 ± 0.002	0.41 ± 0.05
benzaldehyde	1494	1.6 ± 0.34	27 ± 2.5
ethyl benzoate	1644	0.54 ± 0.05	1.2 ± 0.17
methyl salicylate	1753	1.6 ± 0.18	5.4 ± 0.53
β-damascenone	1802	1.2 ± 0.04	8.3 ± 0.19
benzyl alcohol	1837	42 ± 6.2	55 ± 0.99

[a] Concentrations are given as average ± standard deviation ($n = 3$). [b] Quantified on basis of 2-methyl-1-butanol standard.

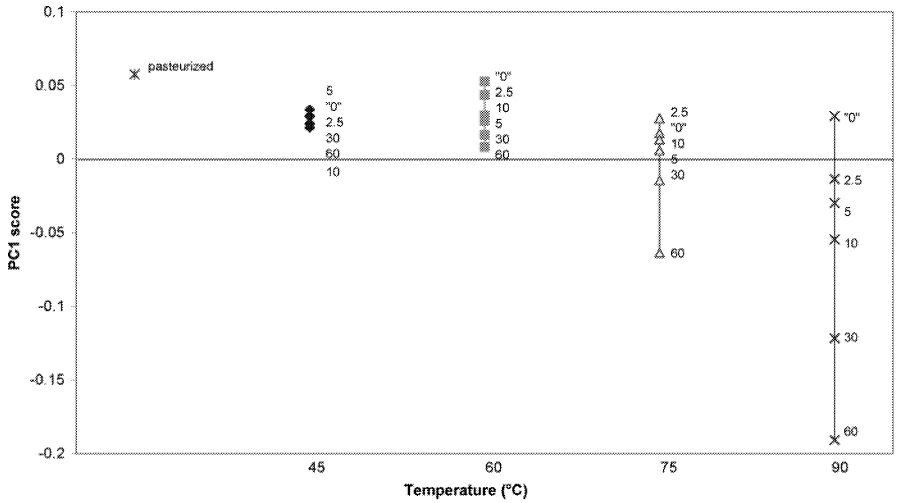

Figure 1. Changes in score values of PC1 during heating of black currant juice: "O" = heated in microwave oven. The numbers 2.5–60 refer to heating times in minutes.

samples heated at 60 °C for 30 and 60 min and at 90 °C for 2.5, 5, 10, 30, and 60 min °C were evaluated against the pasteurized juice. Samples (35 g) were evaluated in 110 mL clear plastic beakers with lids, which were allowed to equilibrate for 1 h before testing. The triangle tests were completely randomized with respect to odd sample and order of presentation within and between judges. The subjects were informed about the principle and task of the test and instructed in the procedure for the evaluation. Within each test, judges were instructed to select the odd sample after smelling the three samples in a given order. The triangle tests were performed during four sessions with seven judges participating, and each judge evaluated the seven tests within the same session. Water was provided to avoid dryness in the throat and to rinse between samples. Assessors were given the opportunity to make comments on the test sheet.

RESULTS AND DISCUSSION

Aroma Changes during Thermal Treatment of Black Currant Juice. The changes in aroma components of black currant juice heated at 45, 60, 75, and 90 °C, respectively, were determined by GC-MS. Concentrations of aroma compounds in the pasteurized and the most extensively heated (90 °C for 60 min) black currant juice are shown in **Table 1**. The concentrations found in pasteurized juice are in the same range as those reported for black currant nectar by Iversen et al. (5), except for 4-terpineol and α-terpineol, for which 100-fold higher levels were found in the present study. The extensive heating decreased the concentration of most esters and gave minor or no decreases in the alcohol concentrations. The concentrations of terpenoids increased with the thermal treatment, except those of linalool and 4-terpineol, which decreased. Aldehyde, furan, phenol, and other compound concentrations increased, many considerably.

Large standard deviations between triplicates were observed for the terpene hydrocarbons α-terpinene, limonene, γ-terpinene, and terpinolene, due to degradation on the Tenax adsorbent material, and for the aldehydes octanal and nonanal due to Tenax artifact formation (*18*). Other compounds could not be quantified, such as cineole, phellandrene, and (+)-3-carene, which were insoluble in the model solution, whereas dimethyl sulfide, diacetyl, methyl acetate, and 2-methyl-3-buten-2-ol was not quantified due to breakthrough on the adsorbent traps. Separation of diacetyl and 2-pentanone was not possible due to coelution.

The average concentrations of the 49 aroma compounds in black currant juice samples (25 in all) heated at the different temperatures (45, 60, 75, and 90 °C) and times were subjected to PCA. A single principal component (PC) explained 52% of the total variation (validated), indicating the temperature effect, whereas the following individual PCs revealed limited variation in the data, although a minor effect of heating time was observed in PC2 (not shown). Sample scores, giving information about the relative importance of each sample, are shown in **Figure 1**. A decrease of sample score value with heating time was generally observed within each temperature group except at 45 °C, where the sample scores were only slightly different (**Figure 1**). The sample scores show that pasteurized juice, all samples heated at 45 and 60 °C, samples heated at 75 °C for "0", 2.5, 5, and 10 min, as well as the 90 °C "0" sample are correlated to each other, having positive PC1 values (**Figure 1**). These samples will in the following be designated the "low processed group". The samples heated at 75 °C for 30 and 60 min and at 90 °C for 2.5, 5, 10, 30, and 60 min are correlated, having negative PC1 values, the two latter samples being the most extreme. These samples will be designated the "high processed group".

The concentration of the majority of the compounds changed relative to the original pasteurized juice during the thermal treatment, which is revealed by the loading variables of PCA that give information about the variables causing the differences between samples (**Figure 2**). All esters are correlated, except methyl butanoate, which influences the model least (**Figure 2A**). The esters are positively correlated to the low processed group, which indicates that the concentrations of esters decrease by the thermal treatment. The terpenoids are correlated to each other and are positively correlated to the high processed group; that is, their concentrations increase by thermal treatment (**Figure 2B**). Exceptions, however, are linalool, 4-terpineol, and rose oxide, which are correlated to each other and are positively correlated to the low processed group, and hence they decrease by thermal treatment. Most of the alcohols are correlated and are positively correlated to the low processed group, and hence decrease during thermal treatment, which is opposite from the development of aldehydes and ketones (**Figure 2C**). Furan

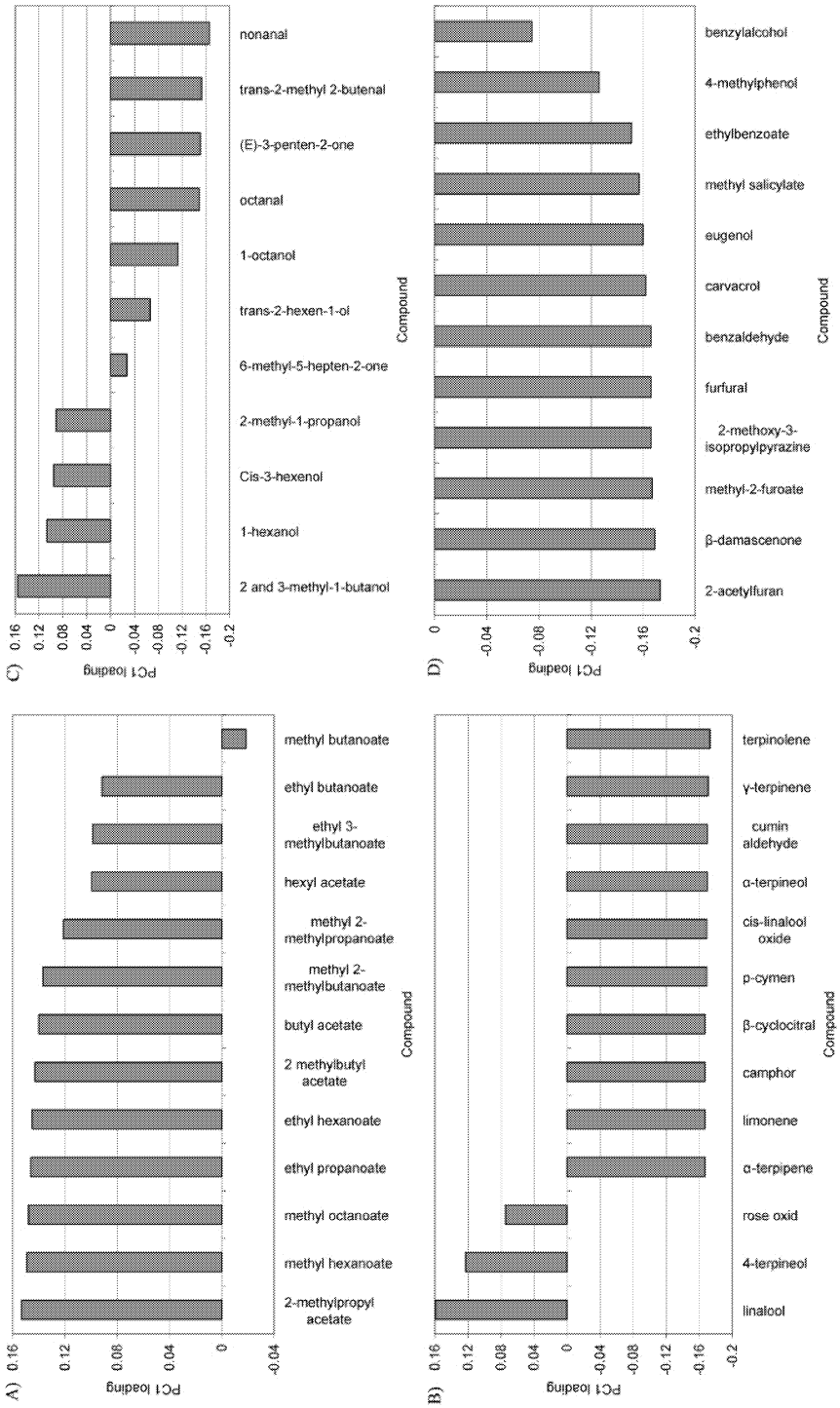

Figure 2. PC1 variable loadings for heating of black currant juice: (**A**) esters; (**B**) terpenoids; (**C**) alcohols and carbonyls; (**D**) others.

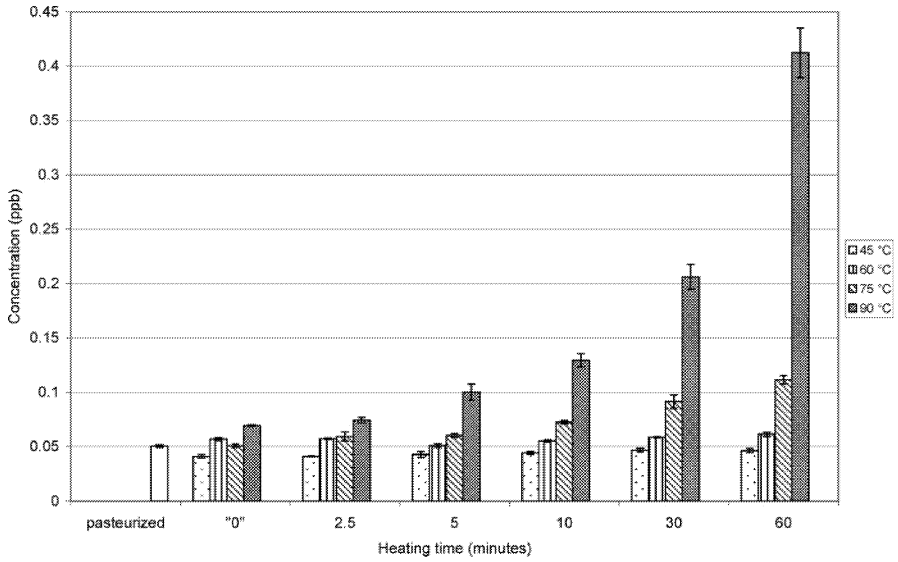

Figure 3. Changes in concentration of 2-methoxy-3-isopropylpyrazine during thermal treatment of black currant juice. Error bars represent standard deviations.

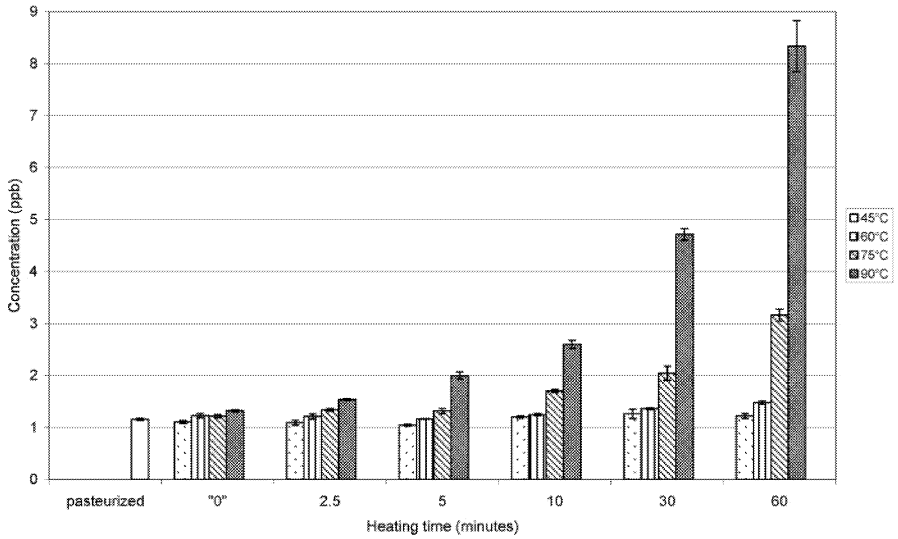

Figure 4. Changes in concentration of β-damascenone during thermal treatment of black currant juice. Error bars represent standard deviations.

derivatives, phenolic, and other compounds (**Figure 2D**) are correlated and are positively correlated to the high processed group; that is, the concentrations increase with thermal treatment.

Some volatile compounds present in black currant juice have been identified by gas chromatography−olfactometry (GC-O) as being more important than others for the aroma. Methyl butanoate, ethyl butanoate, ethyl hexanoate, cineole, 3-methyl-1-butanol, 1-octen-3-one, 2-methoxy-3-isopropylpyrazine, linalool, 4-terpineol, and β-damascenone have been reported by three of four studies to be important for black currant berry and/or juice aroma (*3*, *5*, *7*, *17*). Thermally induced changes in the concentrations of these compounds are therefore expected to influence the odor properties of the juice. The concentrations of 2-methoxy-3-isopropylpyrazine [pea and bell pepper odor (*17*)] and β-damascenone [black currant juice, boiled fruit, flower odor (*17*)] were in the present study found to increase by heating at 75 and 90 °C, with longer heating times resulting in higher concentrations (**Figures 3** and **4**). Pyrazines are formed by Strecker degradation of amino acids (*19*), and thermal treatment of black currants and mango pulp has previously been shown to lead to an increase in the concentration of the carotenoid degradation product β-damascenone (*3*, *20*).

The concentration of linalool [floral odor (*21*)] decreased at temperatures of 75 and 90 °C (**Figure 5**), and the concentration of 4-terpineol [green and moldy odor (*17*)] changed in a similar way, but less pronounced than for linalool (not shown). The

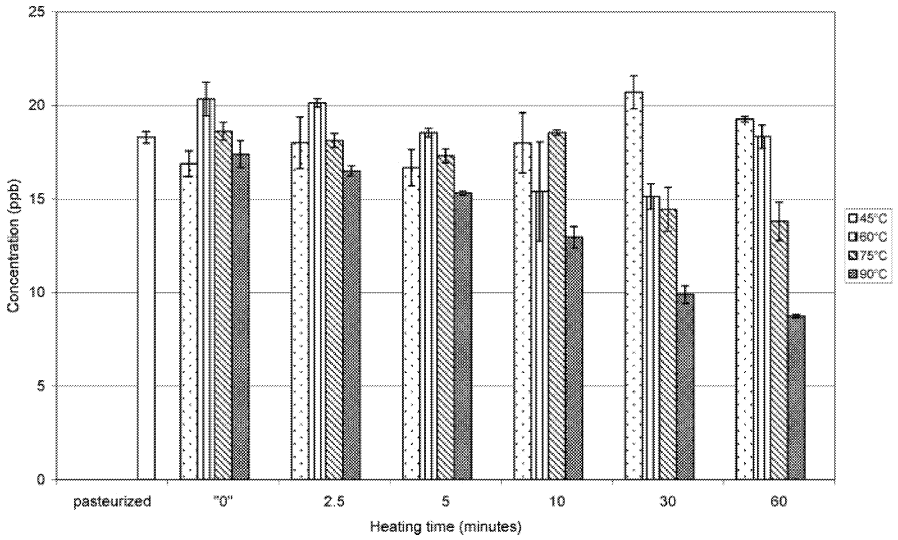

Figure 5. Changes in concentration of linalool during thermal treatment of black currant juice. Error bars represent standard deviations.

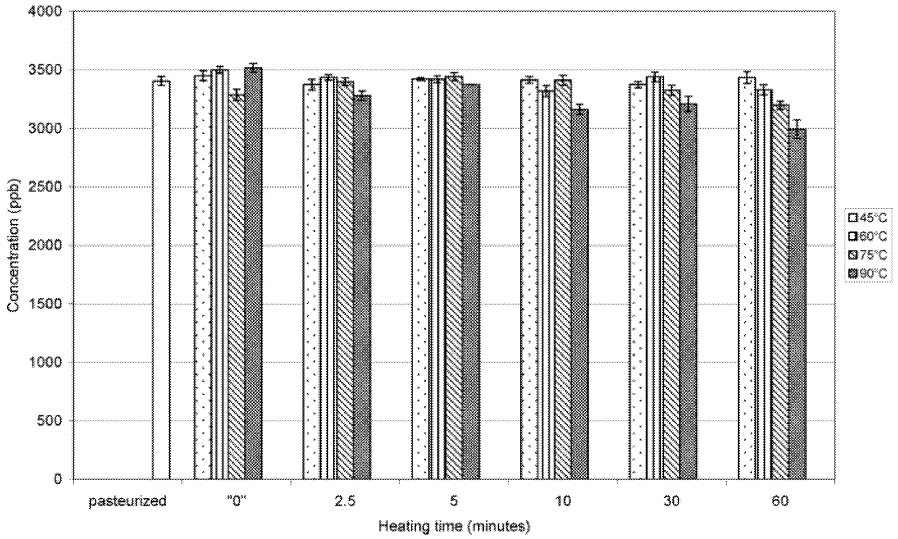

Figure 6. Changes in concentration of 2- and 3-methyl-1-butanol during thermal treatment of black currant juice. Error bars represent standard deviations.

concentration of the other terpenoids increased with the thermal treatment (**Table 1**). von Sydow et al. (*9*) also observed large rearrangements in the monoterpene complex of heated black currant mash. Heat treatment of orange juice has been shown to result in significant terpene hydrocarbon concentration increases (*22*), but in some studies no change or a decrease in the concentration of terpene hydrocarbons has been observed (*9*, *23*, *24*). Terpene hydrocarbons can be formed through acid hydrolysis of terpene alcohols (*25*), and *p*-cymene is formed by oxidation or acid-catalyzed rearrangements of monoterpenes (*26*, *27*). Increases in the concentrations of α-terpineol have been observed upon heating of grape juice, orange juice, and mango pulp (*20*, *28*, *29*). Monoterpenes with hydroxy groups are in part present in plant material as glycosides that can hydrolyze due to low pH, accelerated by heat treatment (*30*), and the presence of monoterpenic and aromatic glycosides has been identified in black currants (*31*). α-Terpineol is in addition a main conversion product of limonene and in particular linalool, these reactions being favored by low pH (*32*, *33*). The observed decrease in linalool concentration can be due to its oxidation to linalool oxide, too, as observed in heated apricot juice (*34*).

Heat had a limited influence on 3-methyl-1-butanol [pungent odor (*21*)] (**Figure 6**) and ethyl hexanoate [fruity odor (*21*)] (**Figure 7**), and the concentration of these compounds decreased at temperatures of 75 and 90 °C. Methyl butanoate and ethyl butanoate [fruity odors (*21*)] behaved correspondingly to ethyl hexanoate, but less distinctly (not shown). Heat increases the rate of acid hydrolysis of esters to the carboxylic acid and alcohol, and the low pH of black currant juice favors many of the chemical reactions that take place during thermal treatment.

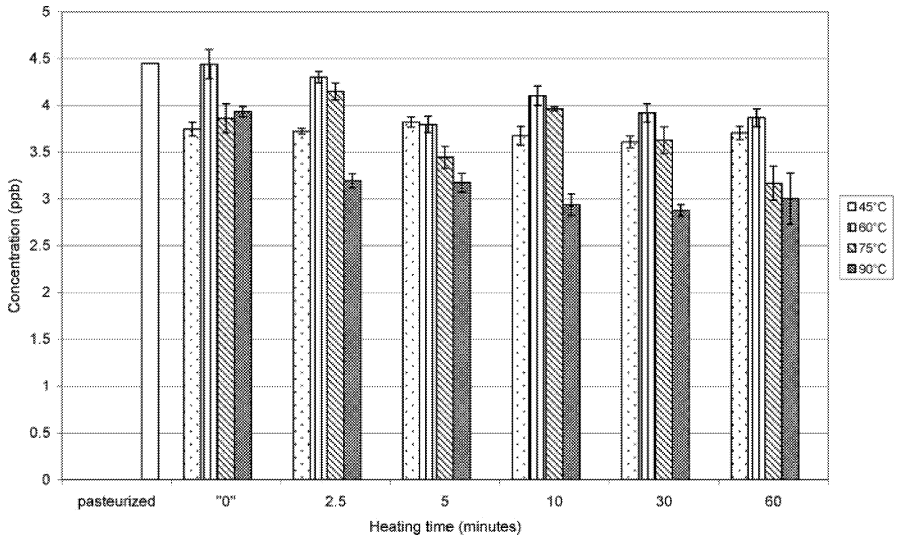

Figure 7. Changes in concentration of ethyl hexanoate during thermal treatment of black currant juice. Error bars represent standard deviations.

Table 2. Triangle Tests of Heated Black Currant Juice

heat treatment	no. of correct answers[a] (*42*)
60 °C for 30 min	13/28 ns
60 °C for 60 min	11/28 ns
90 °C for 2.5 min	17/28 **
90 °C for 5 min	13/28 ns
90 °C for 10 min	17/28 **
90 °C for 30 min	17/28 **
90 °C for 60 min	22/28 ***

[a] All samples are tested against pasteurized juice. ns = nonsignificant, ** and *** indicate significance at $p < 0.01$ and $p < 0.001$, respectively.

The majority of the identified esters decreased during heating in the microwave oven, and at the lower temperatures they remained unchanged even during long heating times (**Figure 7**). Considering the very volatile nature of the esters, the observed loss might partly be due to evaporation during sample handling prior to analysis.

The concentration levels of benzaldehyde, benzyl alcohol, and furanic compounds in the black currant juice increased by heating as has also been observed with tomato juice, and heat-induced formation of furan derivatives is also reported in black currant mash, mango pulp, and orange juice (*8*, *9*, *20*, *29*). In fruit products benzyl alcohol is known to be present as a glycosidic precursor (*35*, *36*), which can be liberated by heat, and furans are derived from the thermal degradation and rearrangement of sugars and ascorbic acid (*37*, *38*). The concentration of phenols increases due to either the thermal degradation of phenolic carboxylic acids or glycosidic release (*39*).

Triangle Tests of Heated Black Currant Juice. Juice samples heated at 60 °C for 30 and 60 min and at 90 °C for 2.5, 5, 10, 30, and 60 min were evaluated against the original pasteurized juice by triangle tests. The odor of black currant juice heated at 60 °C for 30 and 60 min did not significantly differ from that of the pasteurized juice, whereas the odor of juice heated at 90 °C for 2.5, 10, 30, and 60 min significantly differed from the odor of the pasteurized juice (**Table 2**). However, no significant effect of juice heated at 90 °C for 5 min was observed. A possible reason for this discrepancy can be the general strong odor of the juice that might "saturate" the judges' olfactory receptors, or that the sensory differences between the pasteurized sample and the heat-treated samples were minor.

Judges commented that samples heated at 60 °C for 30 and 60 min and at 90 °C for 2.5 and 5 min were very similar to the pasteurized sample and that any difference was difficult to detect. Odors of samples heated at 90 °C for 10, 30, and 60 min were characterized by the judges comments as boiled, sharp, off-flavored, earthy, burned, and less black currant. Results of the triangle tests are in agreement with those of the aroma analyses in which pasteurized juice and 60 °C samples were positively correlated and negatively correlated to samples heated at 90 °C for 2.5, 5, 10, 30, and 60 min (**Figure 1**).

Similar results have been reported by von Sydow et al. (*10*) on triangle tests of unheated black currants and berries heated at 70 °C for 15, 30, and 120 min and for 30 min at 55 and 100 °C. Significant differences among all samples were found, except between 55 °C/30 min and 70 °C/15 min. Odor quality assessment showed that heating led to an increase of odor qualities contributing undesirable aromas to fresh fruit such as cooked odor and sickly, whereas fruity and floral odors decreased. Some odor descriptors appeared in the heated but not in the unheated juice. Brennan et al. (*40*), on the other hand, reported that heating of black currant juice to 85 °C for 2 min significantly increased sweet and natural aroma and black currant and natural flavor.

Implications for Industrial Concentration of Black Currant Juice. Conventional methods for the concentration of black currant juice cause an increase in the levels of phenols, furans, and benzene derivatives (*6*, *7*) as was also observed during the thermal treatments performed in the present study. Compared to the thermal treatments, the losses of esters and alcohols were larger during conventional concentration, and during conventional concentration most terpene compounds decreased in concentration (*7*), whereas thermal treatments resulted in an increase in levels of terpene compounds, except linalool and 4-terpineol. This suggests that aroma changes during conven-

tional concentration are not only thermally induced but can be due to other processing factors such as evaporation or loss through the vacuum pump of the evaporator unit. During 45 °C membrane distillations of black currant juice and a model solution, no changes in aroma composition were detected (*41*).

The heat treatments in the present study were carried out in a closed system to ensure that the observed effects were due to the thermal treatment and not caused by evaporation. The results obtained with the current model system are therefore expected to reflect the chemical changes that are induced by the heating alone, whereas other factors (mainly evaporation) are expected to increase the losses that are observed in specific industrial systems that operate under various conditions and are dependent on the manufacturer.

The results demonstrate that heating of black currant juice at 90 °C, which is within the temperature range used for conventional evaporation of black currant juice, has an effect on the aroma and sensory properties. On the other hand, heating of black currant juice at 60 °C for up to 1 h does not considerably change the aroma profile or the sensory character from that of pasteurized juice. Hence, application of temperatures of 60 °C and lower in, for example, membrane distillation is not expected to cause any heat-induced changes to black currant juice.

ABBREVIATIONS USED

PCA, principal component analysis; PC, principal component; GC-O, gas chromatography−olfactometry; GC-MS, gas chromatography−mass spectrometry; RI, retention index; SD, standard deviation.

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Paper V

Comparison of Tenax TA and Tenax GR as adsorbent materials for headspace sampling of black currant juice aroma

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Varming, C., Andersen, M. L. and Poll, L.

COMPARISON OF TENAX TA AND TENAX GR AS ADSORBENT MATERIALS FOR HEADSPACE SAMPLING OF BLACK CURRANT JUICE AROMA

CAMILLA VARMING, Mogens L. Andersen and Leif Poll

Department of Food Science, Royal Veterinary and Agricultural University, Rolighedsvej 30, 1958 Frederiksberg C, Denmark.

Keywords: Adsorbent material, Tenax, black currant juice, aroma

Abstract
The applicability of Tenax TA and Tenax GR as adsorbent materials for headspace collection of black currant juice aroma was investigated. Higher levels of the terpene alcohols linalool, α-terpineol and 4-terpineol were detected with Tenax TA than with Tenax GR in juice and in model solutions of single terpenes. On the other hand, Tenax GR resulted in higher levels of terpene hydrocarbon breakdown products, indicating that an acid catalysed rearrangement can take place on the surface of the sorbent. Decomposition of terpene alcohols was larger on old than on new Tenax GR. No difference was observed between Tenax TA and Tenax GR for the other types of identified compounds. It is concluded that Tenax TA is more suitable than Tenax GR for determination of terpenes in black currant juice.

Introduction
Tenax TA [poly(2,6-diphenyl-*p*-phenylene oxide)] and Tenax GR (Tenax TA with 30% graphite) are widely used adsorbent materials for headspace collection intended for thermal desorption of volatile organic compounds. These adsorbent materials have high thermal stability and covers collection of compounds with a large range of molecular sizes [1]. Their properties have mainly been studied in relation to analysis of air, whereas only a few studies of food products have been published. Irreproducibility of certain terpenic compounds during the analysis of black currant juice with Tenax GR, lead to the present study of Tenax GR and Tenax TA properties.

Experimental
Materials. Commercial black currant *(Ribes nigrum* L.) juice of the variety Ben Lemond was obtained from an industrial plant. Samples were stored at –18 °C until aroma analyses. Model solutions (glucose conc. 46g/L, fructose conc. 56g/L, citric acid monohydrate conc. 34 g/L in water, 12°Brix) resembling black currant juice were added single terpene standards dissolved in ethanol (final concentration 100 ppb). Two µL of (1000 ppm) single terpene standards in heptane were used for direct injectetion on the traps.

Dynamic headspace collection. 75 g of black currant juice sample or model solution was weighed into a 250 mL glass flask equipped with a purge head. 1.00 mL of internal standard (50 µL/L 4-methyl-1-pentanol, Aldrich, Steinheim, Germany) was added. Sample temperature was equilibrated in a 30°C water bath for 10 min. Under magnetic stirring (200 rpm) the sample was then purged with nitrogen (100 mL/min) for 45 minutes. The volatiles were collected on traps containing 250 mg Tenax TA or Tenax GR (mesh size 60/80, Buchem bv, Apeldoorn, The Netherlands). The collections were

performed on new and old (used 50-100 times) adsorbent material. In order to detect a possible breakthrough of volatiles two identical traps were connected in series.

GC-MS analysis. The collected volatiles were thermally desorbed using an Automated Thermal Desorber (ATD 400, Perkin Elmer, USA) and separation and identification of aroma compounds was carried out on a Hewlett-Packard (Palo Alto, CA) G1800A S GC-MS system equipped with a J & W Scientific DB-Wax column (30 m x 0.25 mm x 0.25 μm). Settings were the same as described by [2]. Identifications were carried out by probability-based matching with mass spectra in the G1035A Wiley library (Hewlett-Packard). Peak areas of aroma compounds were based on single ions converted into total ion areas, and results are presented as relative concentrations = peak area/internal standard area.

Results

Dynamic headspace collection of black currant juice using new and old Tenax GR and TA

New Tenax TA and new Tenax GR were compared by dynamic headspace collection of terpenic compounds from black currant juice. Tenax TA gave slightly higher levels of the terpene alcohols linalool, α-terpineol, and 4-terpineol, that are important constituents of black currant juice aroma (Table 1) [2]. The levels of α-terpinene, myrcene, *p*-cymene and *p*-cymenyl were on the other hand highest with new Tenax GR. No difference was observed between Tenax TA and Tenax GR for the other types of compounds identified (esters, alcohols, carbonyls, phenols and the other terpeneoids) (not shown).

Table 1. Terpenic compounds of black currant juice collected on new and old Tenax TA and GR.

Compound	Tenax GR		Tenax TA	
	Old	New	Old	New
α-pinene	0 ± 0	0 ± 0	117 ± 54	0 ± 0
myrcene	126 ± 11	64 ± 10	24 ± 11	27 ± 14
limonene	379 ± 34	135 ± 80	938 ± 1001	174 ± 77
α-terpinene	1912 ± 190	956 ± 178	165 ± 21	257 ± 114
γ-terpinene	579 ± 61	230 ± 48	66 ± 6	179 ± 88
α-terpinolene	410 ± 42	192 ± 33	52 ± 7	123 ± 54
p-cymene	1580 ± 62	1077 ± 54	382 ± 21	391 ± 44
p-cymenyl	675 ± 42	519 ± 28	0 ± 0	0 ± 0
linalool	129 ± 31	339 ± 61	556 ± 19	478 ± 65
α-terpineol	246 ± 31	402 ± 33	481 ± 8	472 ± 26
4-terpineol	3692 ± 444	5282 ± 318	6356 ± 80	6111 ± 387

Relative concentrations*10000 are given as average ± standard deviation (*n*=5).

New Tenax GR gave higher recovery of terpene alcohols than old Tenax GR, where as levels of terpene hydrocarbons were larger with old than new Tenax GR (Table 1). Only small differences were observed between old and new Tenax TA adsorbent materials. One exception was limonene that partly might be present as a result of background noise. Reactions between monoterpenes and the sorbent surface of Tenax TA has been observed by [3], with higher degradation on old Tenax TA than on fresh. Degradation products identified in that study were also terpenes and aromatic compounds, suggesting rearrangement and dehydrogenation reactions.

A breakthrough of at least 10% on the two adsorbent materials was observed for two esters, one ketone and four alcohols. It was found to be in the same range, except

for ethyl acetate, diacetyl and isobutyl alcohol of which breakthrough was twice as high for Tenax GR as for Tenax TA (not shown).

Collection of single terpenes using new Tenax GR and TA
Dynamic headspace collection of sugar-acid model solutions of single terpenes was carried out in order to further investigate the observed changes (Table 2).

Table 2. Decomposition of single terpenes in model solution collected on new Tenax GR and TA.

Compound	**α-terpineol**		**linalool**		**4-terpineol**	
	Tenax GR	Tenax TA	Tenax GR	Tenax TA	Tenax GR	Tenax TA
α-pinene	38 ± 25	80 ± 109	27 ± 19	241 ± 131	4.5 ± 0.7	3.4 ± 1.0
Myrcene	31 ± 16	0 ± 0	580 ± 49	15 ± 2	0 ± 0	0 ± 0
Limonene	759 ± 346	41 ± 9	245 ± 58	34 ± 12	257 ± 14	178 ± 12
α-terpinene	44 ± 26	0 ± 0	19 ± 7	23 ± 10	179 ± 26	106 ± 57
γ-terpinene	31 ± 19	1.9 ± 0.2	23 ± 5	2.3 ± 0.5	163 ± 19	87 ± 45
α-terpinolene	339 ± 159	36 ± 1	54 ± 14	9.3 ± 0.6	76 ± 9	37 ± 19
p-cymene	138 ± 79	16 ± 2	63 ± 17	9.0 ± 2.5	314 ± 14	63 ± 16
p-cymenyl	182 ± 86	10 ± 0.5	31 ± 10	1.6 ± 0.9	19 ± 2	1.9 ± 0.8
Linalool	0 ± 0	0 ± 0	**1025 ± 301**	**3365 ± 148**	0 ± 0	0 ± 0
α-terpineol	**1039 ± 446**	**1866 ± 28**	16 ± 2	13 ± 1	8.4 ± 0.5	8.0 ± 0.4
4-terpineol	35 ± 10	56 ± 11	9.1 ± 2	0 ± 0	**3214 ± 30**	**3705 ± 145**
Compound	**Limonene**		***p*-cymene**		**α-terpinene**	
	Tenax GR	Tenax TA	Tenax GR	Tenax TA	Tenax GR	Tenax TA
α-pinene	107 ± 177	38 ± 37	59 ± 47	9.4 ± 3.4	67 ± 44	53 ± 44
Myrcene	23 ± 6	19 ± 4	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Limonene	**13186 ± 342**	**12463 ± 646**	52 ± 52	70 ± 8	101 ± 55	94 ± 43
α-terpinene	4.3 ± 7.4	0 ± 0	0 ± 0	0 ± 0	**8883 ± 577**	**9992 ± 658**
γ-terpinene	3.0 ± 5.2	8.3 ± 3.7	0 ± 0	7.4 ± 7.3	25 ± 1	14 ± 5
α-terpinolene	30 ± 8	13 ± 4	0 ± 0	0 ± 0	64 ± 4	25 ± 1
p-cymene	88 ± 15	60 ± 20	**17740 ± 482**	**17274 ± 1834**	4647 ± 75	1940 ± 80
p-cymenyl	83 ± 32	5.0 ± 1.2	45 ± 2	18 ± 7	110 ± 10	17 ± 2
Linalool	5.0 ± 4.4	2.6 ± 4.5	0 ± 0	0 ± 0	0 ± 0	0 ± 0
α-terpineol	0 ± 0	3.6 ±3.6	0 ± 0	0 ± 0	0 ± 0	0 ± 0
4-terpineol	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Relative concentrations*10000 are given as average ± standard deviation (*n*=3).

Higher levels of the single terpene alcohols, particularly linalool were detected with Tenax TA than with Tenax GR, whereas higher levels of the corresponding terpene hydrocarbon breakdown products were detected for Tenax GR (except for α-pinene). **Linalool** was converted mainly to myrcene and limonene (Tenax TA to α-pinene), **α-terpineol** was converted mainly to limonene, α-terpinolene, *p*-cymenyl and *p*-cymene, and **4-terpineol** was converted to *p*-cymene. No differences between the two materials were observed for the analysed single terpene hydrocarbons limonene and *p*-cymene, and these compounds were less prone to degradation than the terpene alcohols. **α-terpinene** on the other hand was oxidised to *p*-cymene on both TA and GR. The results indicate that an acid catalysed rearrangement can take place on the surface of the sorbent [4]. During sampling of a test atmospheric mixture of volatiles, Tenax GR has been shown to have a larger catalytic effect on breakdown than Tenax TA of some compounds [5], and Tenax GR gave irreproducible results and loss of terpenes during determination of terpenes in forest air, probably caused by the presence of graphite in

the adsorbent [6]. On the contrary, Tenax GR gave better yields than Tenax TA for the adsorption of monoterpenes in indoor air monitoring [7]. Also an effect of ozone on Tenax TA has been reported with decomposition of terpenes especially with two or more C-C double bonds [8].

Direct injection of single terpene alcohols on the adsorbent traps gave less pronounced decomposition than after headspace collection (not shown). **Linalool** was converted mainly to myrcene and limonene, **α-terpineol** was converted to limonene and α-terpinolene, and **4-terpineol** was converted to *p*-cymene in agreement with the results from the headspace collections. Likewise, injection on Tenax TA resulted in less decomposition than on Tenax GR. Monoterpene degradation on Tenax TA has been shown to take place during the sample step [3], and changes observed in chromatographic profiles have been explained by interactions between volatile compounds, water and Tenax during headspace collection [9].

It is concluded that Tenax TA is more suitable than Tenax GR for determination of terpenes in black currant juice, al though some decomposition does also occur on Tenax TA.

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Paper VI

Recovery of volatile fruit juice aroma compounds by sweeping gas membrane distillation

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Bagger-Jørgensen, R., Meyer, A. S., Varming, C. and Jonsson, G.

Recovery of volatile fruit juice aroma compounds by sweeping gas membrane distillation

Rico Bagger-Jørgensen[a,c], Anne S. Meyer[a], Camilla Varming[b], Gunnar Jonsson[c*]

[a] Food Biotechnology & Engineering Group, BioCentrum-DTU, Building 221, Technical University of Denmark, 2800 Lyngby, Denmark

[b] Department of Dairy and Food Science, The Royal Veterinary and Agricultural University, DK-1958 Frederiksberg C, Denmark

[c] Department of Chemical Engineering, Technical University of Denmark, Building 229, 2800 Lyngby, Denmark

Abstract

The influence of feed temperature (10 – 45°C), feed flow rate (300 – 500 L/h) and sweeping gas flow rate (1.2–2 m^3/h) on the recovery of fruit juice aroma compounds by sweeping gas membrane distillation (SGMD) was examined on an aroma model solution and on black currant juice in a lab scale membrane distillation set up. The flux of SGMD increased with an increase in temperature, feed flow rate or sweeping gas flow rate. An increase in temperature and feed flow rate also increased the concentration factors ($C_{permeate}/C_{feed}$) of the aroma compounds. At 45°C the highly volatile and hydrophobic aroma compounds obtained the highest concentration factors: 12.1–9.3 (black currant juice), 17.2-12.8 (model solution). With black currant juice a volume reduction of 13.7 vol.% (45°C, 400 L/h) resulted in an aroma recovery of 73-84 vol.% for the highly volatile compounds. The theoretically predicted aroma recovery values were in accordance with the experimentally obtained values. Compared with vacuum membrane distillation (VMD) the SGMD technique was demonstrated to give lower water permeability. Aroma recovery with SGMD was less influenced by the rate of feed flow but more influenced by the temperature than VMD. Although not as efficient as VMD in lab scale experiments, aroma recovery by SGMD deserves further consideration as an alternative technique for gentle aroma stripping in fruit juice processing.

Keywords: Sweeping gas membrane distillation, vacuum membrane distillation, black currant juice, fruit juice aroma, aroma recovery

[*] Corresponding author: Fax: (+45) 45 88 22 58, e-mail: gj@kt.dtu.dk

1. Introduction

Sour cherries and black currants are two of the main fruit crops in Denmark and are important fruit crops in other European countries as well. Both of them contain large amounts of natural antioxidants like phenolic compounds and vitamin C, black currants in particular (Grassin & Fauquembergue, 1996; Macheix et al., 1990).

Black currant juice and cherry juice each have a unique aroma profile that comprises a diverse mixture of volatile aroma compounds representing different chemical groups. More than 120 aroma compounds have been identified in black currant juice, the main groups constituting terpenes, esters and alcohols (Leino & Kallio, 1993; Kollmansberger & Berger, 1994; Iversen, Jakobsen, & Olsen, 1998; Varming & Poll, 2003) whereas about 90 aroma compounds have been identified in sour cherry juice, the main groups constituting alcohols and terpenes (Schmid & Grosch, 1986; Poll & Lewis, 1987). The last stage in fruit juice processing is concentration, where the juice volume is reduced to a solids content of 65-75% (Śulc, 1984). The most widely used concentration method in the juice industry today involves multi-falling film evaporation, where the temperature in the concentration unit can reach 105°C (Varming & Poll, 2003). The aroma fraction is removed from the concentrate in the first step of the evaporation process and is later added back to the juice concentrate prior to reconstitution of the juice (Śulc, 1984). Thermal treatment of fruit juice, including the high temperatures employed during the aroma recovery, has been reported to result in undesirable changes in the aroma profile of black currant juice (Mikkelsen & Poll, 2002; Varming & Poll, 2003). These changes in turn are expected to negatively influence the sensory impression of the final, reconstituted juice (Varming & Poll, 2003). Heat treatment of black currants has also previously been reported to cause undesirable odour of the berries (Sydow & Karlsson, 1971a,b). During a conventional, fractional distillation process of black currant juice only about 50-75% of the volatiles of the base juice are recovered in the aroma concentrate. A significant source of loss is the singlings, i.e. the evaporated water fraction that is discarded during the processing (Varming & Poll, 2003). However, losses of esters, alcohols, carbonyls and terpenes also occur during the other processing steps (Mikkelsen & Poll, 2002).

The development of membrane distillation (MD) techniques could contribute to solve the problems related to the concentration process. The principle of separation by MD is based on separation of an aqueous feed solution and a permeate fraction by means of vaporization and diffusion of vapour through the gas phase inside the pores of a micro-porous hydrophobic membrane. The driving force of the process is the partial pressure gradient between the two membrane sides (Couffin, Cabassud & Lahoussine-Turcaud, 1998). The hydrophobic micro-porous membrane only acts as a support for the liquid-vapour interface and does not contribute to the separation mechanism (Mulder, 1996). The MD process can be carried out with different experimental configurations, depending on the method by which the vapour is recovered from the membrane pores. Vacuum membrane distillation (VMD) and sweeping gas membrane distillation (SGMD) represent two methods in which the permeate fraction must be collected in an external unit. In SGMD a collecting gas sweeps the vapour, whereas in VMD the vapour is removed by a vacuum system (Khayet, Godino & Mengual, 2002). The advantage of these configurations is that they combine a relative low (SGMD) or negligible (VMD) conductive heat loss with a reduced mass transfer resistance (Bandini, Saavedra & Sarti, 1997; Khayet, Godino & Mengual, 2000). Besides this, high partial pressure gradients and consequently high fluxes are created in VMD because of a permeate pressure much lower than the vapour pressure of the

diffusing species (Lawson & Lloyd, 1996; Izquierdo-Gil & Jonsson, 2003). The most important heat and mass transfer resistances are located in the boundary layers near the membrane and can be correlated with the operating parameters (Calabrò, Jiao & Drioli, 1994).

In a previous paper we examined the workability of the VMD principle for recovery of black currant juice aroma from black currant juice (Bagger-Jørgensen et al., 2004). In the present paper the aim is to study the potential of the SGMD configuration for recovering black currant and cherry juice aroma compounds from a model solution and to compare results obtained by SGMD and VMD at the same parameter settings. The influence of the sweeping gas flow rate (SGMD only), feed temperature and feed flow rate on the permeate flux and the concentrations factors of 12 selected aroma compounds were examined in laboratory scale set-ups. Besides a model aroma solution resembling both black currant and cherry juice (same sugar and acid level) genuine black currant juice was used as feed. The model solution contained 12 selected aroma compounds that are characteristic for black currants juice and/or cherry juice aroma (Figure 1). Ethyl butanoate, isoamyl acetate, *cis*-3-hexen-1-ol, 3-methyl-1-butanol, 1,8 cineole, linalool, 4-terpinenol, β-damascenone and eugenol are important for black currant juice aroma as determined by GC-sniffing of black currant juice and nectar isolates (Iversen et al., 1998; Varming, Petersen, & Poll, 2004). Benzaldehyde, diacetyl, linalool and eugenol are important aroma compounds in sour cherry fruit and/or juice as determined by GC-sniffing of aroma isolates or by odour threshold values (Schmid & Grosch, 1986; Poll & Lewis, 1987). Furfural was selected to indicate heat treatment. The aroma components used in the model solution differ according to their molecular structure, boiling point, solubility, hydrophobicity (Log P) and relative volatility (Table 1).

2. Materials and methods

2.1 Model solution

The aqueous model solution was developed to resemble a fruit juice and had a glucose content of 15% weight/volume, a citric acid content of 1.5% weight/volume, and an ethanol content of 1% volume/volume. pH was adjusted to 3 with potassium hydroxide. Sorbic acid potassium salt (corresponding to 1000 ppm sorbate) was added as a preservative. The model solution was kept frozen (at -20°C) in portions of 5 L until use. Prior to SGMD experiments the model solution was thawed at room temperature (20°C). The 12 aroma compounds (figure 1) were added each to a final concentration of 1 ppm and the solution was then heated to the experimental temperature.

2.2. Black currant juice

The black currant juice had a sugar level corresponding to 16 ^{o}Brix and was obtained from an industrial juice processing plant (Vallø Saft A/S, Vallø, Denmark). Prior to sampling the juice had been processed as described by Bagger-Jørgensen et al. (2004). The juice was kept frozen (at -20°C) in portions of 5 L until use. Prior to MD experiments the juice samples were thawed at room temperature (20°C) and heated to the experimental temperature immediately prior to use.

2.3 Experimental set-ups

The experimental set-up of the VMD system was as described previously (Izquierdo-Gil and Jonsson, 2003). The membrane module had one canal and one flat PTFE (polytetrafluoroethylene) membrane that had a total membrane surface area of 37 cm^2 and a pore size of 0.1 μm.

The schematic representation of the SGMD set-up is shown in figure 2. The membrane module had 24 canals and the total membrane surface area was 159 cm^2. In all experiments a flat polytetrafluoroethylene (K150) membrane (thickness: 260 µm) with support and a pore size of 0.1 µm was used (Osmonics, Minnetonka, USA). The reservoir volume was approximately 3.3 L. The feed side composition and temperature were considered as constant within the membrane module. The experimental temperature was taken as the average of the inlet and outlet temperature at the feed side of the membrane module. A maximum temperature drop of approximately 1°C between inlet and outlet was observed. The sweeping gas flow was counter-current to the feed flow. The sweeping gas used was atmospheric air with an inlet temperature of 27°C ± 1°C and a flow rate of either 2 m^3/h (5.8 m/s) or 1.2 m^3/h (3.5 m/s). The membrane flux was measured by timing the collection of permeate in the condensation trap refrigerated with ethanol (0-2°C) and by weighing the permeate fraction. The influence of the feed temperature (10°C – 45°C) and the feed flow rate (100 L/h (only VMD), 300-500 L/h) on the performance of VMD and SGMD were examined. All data presented are averages of at least 3 measurements. Data were compared and grouped after one-way analysis of variances (one way ANOVA, $p < 0.05$) using the statistical program MINITAB Release 12 (Minitab Statistical Software, Addison-Wesley, Reading, MA).

2.4 Dynamic headspace collection and *GC-MS analysis*

In the experiments where genuine black currant juice was used, the aroma of the initial juice samples, the distilled samples and the final retentate fractions were collected by dynamic headspace sampling and analysed by GC-MS. Samples were thawed, and 75 mL of juice or 5.0 mL of aroma distillate was transferred to a 250 mL glass flask equipped with a purge head. Aroma distillates were diluted with 70 mL of water. 1.00 mL of internal standard (50 µL/L 4-methyl-1-pentanol, Aldrich, Steinheim, Germany) was added to each sample. The sample was then placed in a water bath at 30°C, and the temperature aligned for 10 min. Under magnetic stirring (200 rpm) samples were purged with nitrogen (100 mL/min) for 45 min. Volatiles were collected on traps containing 250 mg Tenax-TA (mesh size = 60/80, Buchem bv, Apeldoorn, The Netherlands). Headspace collections of juice were performed in triplicates. Samples were then subjected to automated thermal desorption and GC-MS analysis according to the procedure described previously (Bagger-Jørgensen et al., 2004).

2.5 Dynamic headspace collection and GC-FID analysis

Dynamic headspace GC analyses employing flame ionisation detection was used for analysing all samples resulting from the experiments with the model solution. Samples were thawed, and 50.0 g of model solution or 5.0 g of aroma distillate were weighed into a 150 mL glass flask equipped with a purge head. Each sample was diluted with water to 100 g, and 1.00 mL of an internal standard (50 µL/L 4-methyl-1-pentanol, Aldrich, Steinheim, Germany) was added. Each sample was placed in a water bath at 30°C and incubated for 15 min. The samples were purged with nitrogen (100 mL/min) for 15 min. Volatiles were collected on Tenax-TA traps (225 mg) with mesh size 60/80 (Varian, Inc., USA). The headspace collections on model solution samples were performed in triplicates. The collected volatiles were thermally desorbed using an Automated Thermal Desorber (ATD 400, Perkin Elmer, USA). Desorption time from the trap to the cold trap (-30°C) was 3 min at 25°C, with a helium flow of 75 mL/min. Volatiles were

desorbed from the cold trap to the GC-column by flash heating from -30°C to 250°C (1 min.). Separation and identification of aroma compounds were carried out on a Hewlett-Packard (Palo Alto, USA) 5890 System (GC-FID) equipped with a J & W Scientific DB1701 column (30 m x 0.53 mm x 1.00 µm) using helium as the carrier gas (8 mL/min). The GC-FID split ratio was 8:18. The column temperature was kept at 35°C for 1 min, increased by 4 °C/min to 175°C, increased by 10 °C/min to 250°C and kept isothermally for 1 min. The FID temperature was 250°C. The gas flow of air, helium and make-up were 400 mL/min, 30 mL/min, and 22 mL/min respectively. Identifications were carried out by comparisons with mass spectra and retention times of authentic reference compounds. The computer program HP GC Chemstation (Rev. A. 06.03[509], Hewlett-Packard 1990-1980) was used to calculate the peak areas. Results are given as relative concentrations (peak area divided by internal standard area).

The mass balances were calculated as percentage recoveries, i.e. the sum of volatiles in the final retentate and the collected permeate divided by the level of volatiles found in the initial feed, and then times 100: $[(C_{retentate} + C_{permeate})/C_{feed}] \times 100$. (Bagger-Jørgensen et al., 2004). The concentration factor of each aroma compound was calculated as the ratio between the concentration of the aroma compound in the permeate and the concentration of the aroma compound in the feed initially: $(C_{permeate})/C_{feed})$ (Bagger-Jørgensen et al., 2004).

3. Results

3.1 Mass balances

To be able to evaluate the aroma recovery the mass balance of each aroma compound must be approximately 100%. In all experiments the total recovered amounts of each aroma compound were satisfactory as they ranged from 89% to 110% (data not shown). The amount of furfural did not increase significantly at 45°C because the constituents that can form furfural were not present in the model solution.

3.2.Concentration factors: Model solution

An increase in the SGMD feed flow rate from 300 L/h to 500 L/h, at the constant feed inlet temperature of 30°C, resulted in a significant increase in the concentration factors (CFs) of the aroma compounds ethyl butanoate, isoamyl acetate and 1,8-cineole. Most of the other compounds also seemed to increase, but not significantly (Table 2). At the highest flow rate of 500 L/h the CFs ranged from 3.2 to 12.5. 1,8-Cineole had the highest CF followed by linalool, isoamyl acetate and ethyl butanoate. *cis*-3-Hexen-1-ol had by far the lowest concentration factor while the other aroma compounds had CFs between 5.2 and 8 (Table 2).

An increase in the SGMD temperature from 10°C to 45°C, at the constant feed flow rate of 400 L/h, resulted in a significant increase in the CFs of all aroma compounds except eugenol (Table 3). Ethyl butanoate and 1,8-cineole were, however, the only compounds, whose CFs increased significantly with each temperature increase step from 10°C to 45°C (Table 3). Within the parameter settings examined the highest concentration factor of each aroma compound was obtained at the highest temperature, 45°C, where the CFs ranged from 3.6-16.3 (Table 3). 1,8-Cineole had the highest CF followed by isoamyl acetate, ethyl butanoate and linalool. *cis*-3-Hexen-1-ol had by far the lowest concentration factor while the other aroma compounds had CFs between 6.3 and 9.6 (Table 3).

A reduced sweeping gas flow rate of 1.2 m^3/h (3.5 m/s) (SGMD), at constant temperature (45°C) and feed flow rate (400 L/h), did not significantly change the concentration factors (Table 3). With the reduced gas flow rate the CFs thus ranged from 3.8-17.2 (Table 3). As with the sweeping gas flow rate of 2 m^3/h (5.8 m/s) it was 1,8-Cineole that had the highest CF followed by ethyl butanoate and isoamyl acetate. *cis*-3-Hexen-1-ol and eugenol had the lowest CFs while the other aroma compounds had CFs between 7.0 and 10.6 (Table 3).

An increase in the VMD feed flow rate from 100 L/h to 500 L/h, at the constant feed inlet temperature of 30°C, resulted in a significant increase in the CFs of all aroma compounds except eugenol (Table 4). An increase in the feed flow rate from 300 L/h to 500 L/h also resulted in a significant increase in the CFs of all aroma compounds except for 4-Terpinenol and eugenol (Table 4). At the highest flow rate of 500 L/h the CFs ranged from 6.9 to 25.1. Isoamyl acetate and ethyl butanoate had highest CFs followed by 1,8-cineole (Table 4).

A decrease in the VMD temperature from 45°C to 10°C, at the constant feed flow rate of 400 L/h, resulted in a significant increase in the CFs of all aroma compounds except 4-Terpinenol (Table 4). Hence, within the parameters settings examined, the highest concentration factor of each aroma compound was obtained at the lowest temperature of 10°C, the only exception being 4-Terpinenol. The CFs ranged from 7.6 to 47.0. Isoamyl acetate and ethyl butanoate had the highest CFs followed by 1,8-cineole while 4-Terpinenol had the lowest (Table 4).

3.3 Concentration factors: Black currant juice as feed

An increase in the SGMD temperature from 30°C to 45°C, at the constant feed flow rate of 400 L/h, resulted in a significant increase in the CFs of isoamyl acetate, ethyl butanoate, 1,8-cineole and 4-Terpinenol. The increase in the CF of *cis*-3-hexen-1-ol with the increase in temperature did, not reach statistical significance, however (Table 5). At the highest temperature of 45°C the CFs ranged from 4.0 to 12.1. 1,8-Cineole had the highest followed by ethyl butanoate and isoamyl acetate while *cis*-3-hexen-1-ol had the lowest (Table 5).

A decrease in the VMD temperature from 30°C to 10°C, at the constant feed flow rate of 400 L/h, resulted in a significant increase in the CFs of isoamyl acetate, ethyl butanoate, 1,8-cineole and *cis*-3-hexen-1-ol. The CFs of 4-Terpinenol did, however, not increase significantly (Table 5). At the highest temperature of 45°C the CFs ranged from 7.4 to 31.3. Isoamyl acetate and ethyl butanoate had the highest CFs followed by 1,8-cineole while 4-Terpinenol had the lowest (Table 5).

3.4 Volumetric amounts recovered

In the SGMD experiments with black currant juice the extent of aroma recovery differed markedly among the selected five aroma compounds. The amount of recovered aroma, at a 13.5% feed volume reduction, varied between 83% and 35% (45°C, 400 L/h) (Figure 4, exp. data). The highest level of recovered aroma was obtained for 1,8 cineole (83%) followed by ethyl butanoate (79%) and isoamyl acetate (72%).

In the VMD experiments with black currant juice the extent of aroma recovery also differed markedly among the selected five aroma compounds. Thus, the amount of recovered aroma, at a 4.5% feed volume reduction, varied between 79% and 31% (10°C, 400 L/h) (Figure 5, exp. data). The highest level of recovered aroma was obtained for isoamyl acetate (79%) followed by ethyl butanoate (75%) and 1,8 cineole (66.5%).

3.5 Permeate flux

As expected, the SGMD flux was significantly influenced by the parameter settings. A significant increase in permeate flux was thus seen with increased temperature from 10°C to 45°C, feed flow rate from 300 L/h to 500 L/h (Tables 2 and 3) and sweeping gas flow rate from 1.2 m^3/h to 2 m^3/h (Figure 3, 30°C and 45°C). An increase in temperature had the largest influence on the level of the permeate flux and the highest permeate flux of 5.04 L/h/m^2 was obtained at 45°C (400 L/h) (Table 3).

The VMD flux was also significantly influenced by the parameter settings. A significant increase in the permeate flux was thus seen with an increase in the temperature from 10°C to 45°C and an increase in feed flow rate from 300 L/h to 400 L/h. The permeate flux did, however, not increase when the feed flow rate was increased from 400 L/h to 500 L/h. The temperature had the largest influence on the level of the permeate flux and the highest permeate flux of 48.55 L/h/m^2 was obtained at the highest feed temperature of 45°C (feed flow 400 L/h) (Table 4). The kind of feed used (model solution or black currant juice) did not have a statistical significance ($P<0.05$) on the permeate flux obtained with the MD techniques SGMD and VMD (Table 2, 3, 4 and 5).

3.6 Membrane permeability

The water vapour pressure and thus water transport through the membrane depends on the temperature at the membrane surface. The relationship between flux and the water vapour pressure difference of the feed and permeate in VMD is shown in figure 3 (solid line). The correlation between the permeate flux and the water vapour pressure difference (P_{feed} - $P_{permeate}$) was practically linear in the range of operative conditions with $y = 0.007471x$ and $R^2 = 0.9990$, which signified that the difference in water vapour pressure was the real driving force across the membrane in VMD.

The water vapour pressure at the permeate side ($P_{permeate}$) could not be measured in SGMD and was thus calculated theoretically. The permeate flux depicted as a function of the water vapour pressure difference (P_{feed} - $P_{permeate}$) in SGMD is shown in figure 3 (dotted line). The correlations were practically linear in the range of operative conditions with $y = 0.0008269x$ and $R^2 = 0.9951$.

At the experimental conditions used (30°C, 400 l/h) the influence of the temperature polarization at the feed side was small (Table 2 and 4). For this reason the temperature polarization was neglected in the above correlations.

4. Discussion

4.1 SGMD with the model solution as feed

The feed flow rates examined were 300 L/h (0.8 m/s), 400 L/h (1.1 m/s) and 500 L/h (1.4 m/s). These flow rates correspond to Reynolds numbers of approximately 1350 to 2250. These Reynolds numbers signify that the flow was mainly in the laminar region. An increase in the experimental feed flow rate from 300 L/h to 500 L/h only resulted in a 6 % increase in the permeate flux (Table 2). This limited effect of the feed flow rate on the permeate flux agrees well with the data reported by Khayet, Godino & Mengual (2003) where the influence of feed flow rates was negligible in the range 0.2 m/s – 1.2 m/s.

The feed flow rate had, however, a pronounced influence on the concentration of the aroma compounds in the permeate. Hence, the permeate concentration of all the aroma compounds examined had a tendency to increase with increasing feed flow rate. The increase in the

concentration factors was statistically significant for the two esters ethyl butanoate, isoamyl acetate and the terpene 1,8-cineole. Together with the terpene linalool these three aroma compounds were the ones that obtained the highest concentration factors (Table 2). The finding that these compounds were mostly affected by alterations in the feed flow rate is not surprising considering that ethyl butanoate, isoamyl acetate and 1,8-cineole all are hydrophobic and highly volatile and compared to 4-terpinenol the last terpene in the model solution 1,8-cineole and linalool have lower boiling points and aqueous solubility (Table 1).

Figure 6 illustrates the transmembrane temperature, partial pressure and concen-tration gradient in SGMD. Throughout the SGMD process the vapour-liquid equilibrium conditions at the membrane-feed boundary in the liquid phase will differ from the bulk condition: The temperature at the membrane surface will decrease because of the temperature polarisation effect and the evaporation at the vapour-liquid interface (solid line: T_{mem}, Figure 6). Concentration polarisation is also present, due to the mass flux across the membrane the aroma concentration at the membrane surface will decrease below the level in the bulk (solid line: C_{mem}, Figure 6). Temperature and concentration polarisation consequently decrease the driving force (partial pressure difference) (Khayet et al., 2003). An increase in the feed flow rate increases the heat transfer coefficient of the aroma compounds in the boundary layer and thus reduces the temperature polarization, which results in a higher temperature at the membrane surface (T_{mem}: From the solid to the dotted line, Figure 6) and consequently a higher flux. The increase in feed flow rate had, however, only a minor effect on the permeate flux, since the driving force and thus the required amount of heat for evaporation are low in SGMD. An increase in the feed flow rate increases the mass transfer coefficient of the aroma compounds in the boundary layer and thus reduces the concentration polarization: The concentration of the aroma compounds at the membrane surface will consequently increase (C_{mem}: From the solid to the dotted line, Figure 6), and this will in turn result in a higher permeate concentration ($C_{permeate}$: From the solid to the dotted line, Figure 6). Concentration polarization at the feed side was not very significant in SGMD because of the low aroma fluxes and concentration factors obtained (Table 2 and 3). The experimental results were, as expected, in accordance with the above as the concentrations factors of the aroma compounds and the permeate flux increased with increased feed flow rate (Table 2). Figure 7 illustrates the temperature and concentration gradients along both sides of the membrane in SGMD (Khayet et al., 2000; Rivier et al., 2002). In the experimental set-up the sweeping gas flow was counter-current to the feed flow (Figure 7). The temperature (T_f) and concentration (C_f) of aroma compounds in the feed was considered constant within the membrane module. The temperature (T_{mf}) and concentration (C_{mf}) at the membrane surface were slightly lower than in the feed because of the temperature and concentration polarization. The temperature of the gaseous permeate will increase along the membrane when $T_{gas} < T_{feed}$ and the temperature at the membrane-permeate surface (T_{mp}) will thus be higher than the temperature in the gas phase (T_p). Because of diffusion resistance in the membrane-permeate boundary layer the concentration of the aroma compounds at the membrane surface (C_{mp}) will be slightly higher than in the gas phase (C_p). C_{mp} and C_p will increase along the membrane as the degree of saturation of the sweeping gas increases. Saturation of the gas phase will in turn increase the partial pressure in the saturated region and hence decrease the aroma flux. Within the range of the feed flow rate examined most of the aroma compounds gave a maximum concentration factor at 500 L/h. With a further increase in feed flow rate, it might be possible to obtain higher concentration factors especially for the highly volatile aroma compounds. An increase in feed

flow rate above 500 L/h would probably not result in a higher permeate flux. Khayet, et al. (2000) have reported a negligible effect of the feed flow at turbulent flows corresponding to Reynolds numbers of 3270 and 8800.

Transport through dense films may be considered as an activated process, which can be represented by an Arrhenius type of equation (Equation 1). This implies that the temperature may have a large effect on the transport rate (Mulder, 1996).

$$J_i = J_o e^{\frac{-Ea}{RT}} \qquad (1)$$

Where J_i is the flux of compound i, J_o is a temperature independent constant, E_a is the activation energy, R is the gas constant and T is the temperature. An Arrhenius plot is illustrated in figure 8 where the natural logarithm (ln) to the saturated pressure (P^{sat}), as a function of the reciprocal temperature (1/T) is shown for water and the aroma compounds ethyl butanoate and 3-methyl-1-butanol (solid lines). The slopes of the lines resemble the energy of activation (E_a) with $|E_{a,3\text{-methyl-1-butanol}}| > |E_{a,water}| > |E_{a,ethyl\ butanoate}|$. The saturated pressure of 3-methyl-1-butanol is, therefore, more influenced by a change in temperature than water, which is more influenced than ethyl butanoate. It is, however, not possible to see this difference in the results, since the partial pressure of a compound in the membrane-feed interface besides the saturated pressure also depends on the molar fraction in the liquid phase and the activity coefficient. The more hydrophobic a compound is (Table 1) the higher is its activity coefficient and thus its volatility in a diluted solution.

An increase in the feed temperature in SGMD resulted in an exponential increase in the permeate flux (Table 3), which may be explained by the Antoine equation, which predicts an exponential relationship between the driving force (partial pressure difference) and temperature (Lawson and Lloyd, 1997). As illustrated in figure 6: The partial pressure of a component in the membrane-feed interface ($P_{i,mem}$) increases with the temperature (from the solid line to the dotted line), which increases the transmembrane partial pressure difference and consequently the flux. An increase in temperature will increase the diffusivity of the aroma compounds in the feed resulting in an increase in the flux of the aroma compounds (Garcia-Payo, Rivier, Marison & von Stockar, 2002). The temperature and concentration polarization at the feed side will also be influenced by an increase in temperature. As the heat lost though the membrane itself by conduction and the amount of heat to vaporise the permeate is increased, the temperature in the membrane-feed boundary layer will decrease and the temperature polarisation will thus increase. The higher permeate flux obtained will decrease the aroma concentration in the membrane-feed boundary layer and thus increase the concentration polarisation of the aroma compounds, which may limit the increase in permeate concentration. The significant increase in the permeate flux with an increase in temperature may result in a higher degree of saturation of the permeate, which will increase the permeate pressure and thus limit the flux increase. The degree of aroma concentration obtained in the permeate is determined by the ratio between the water flux and the flux of the aroma compounds, both will increase with temperature. The experimental results were in agreement with the above: The permeate flux at 45°C was almost 19 times higher than at 10°C. All the aroma compounds obtained the highest concentration factor at the highest experimental temperature (45°C), which means that the flux of the aroma compounds must have increased more than the water flux when temperature was increased from 10°C to 45°C. Ethyl

butanoate, isoamyl acetate and 1,8-cineole obtained the highest CFs of all the aroma compounds examined (Table 3). Eugenol was the only compound, which did not increase significantly with a temperature increase from 10°C to 45°C. A reason may be that eugenol had the highest molecular weight and the highest boiling point of the aroma compounds examined (Table 1 and 3). An increase in temperature to 60°C, to obtain higher concentrations factors and a higher permeate flux, might be possible in the concentration of cherry and black currant juice, but higher temperatures may initiate heat induced deterioration of sensory attributes and nutritional value (von Sydow & Karlsson, 1971a,b).

With the experimental set-up used the maximal flow rate of sweeping gas, as used in the feed flow rate and the temperature experiments, was 2 m^3/h (5.8 m/s). A series of experiments at a lower gas flow rate (1.2 m^3/h (3.5 m/s)) were conducted to investigate the influence of the gas flow rate. An increase in the sweeping gas flow rate significantly increased the permeate flux at 30°C and 45°C (Figure 3). An explanation for this result may be that the thickness of the membrane-gas boundary layer and the saturation degree of the permeate decreased (Garcia-Payo et al., 2002), which resulted in a reduced permeate pressure and consequently an increase in the pressure gradient across the membrane. The increase in permeate flux and aroma flux with increased sweeping gas flow rate may, however, be limited because it results in an increased saturation of the permeate and consequently an increase in the permeate pressure. An increase in the sweeping gas flow rate from 1.2 m^3/h to 2 m^3/h did not increase the CFs of the aroma compounds at 45°C (Table 3). An explanation could be that the increase in the sweeping gas flow rate reduces the water and the aroma saturation of the gas phase with the same degree resulting in an unaltered ratio between them. Since the sweeping gas is partly saturated, an increase in the flow rate to a level above 2 m^2/h would probably lead to an increase in the permeate flux.

This study proved the workability of the SGMD principle for recovery of black currant and cherry juice aroma from a model solution. At 45°C SGMD results in a minimum of un-desirable thermal changes compared with the falling film evaporation technique, which often takes place above 100°C. With 45°C and a feed flow rate of minimum 500 L/h (1.4 m/s) a satisfactory degree of recovery may be achieved. A further concentration of the permeate is, however, necessary to reduce the volume.

4.2 SGMD vs. VMD

There was a significant difference in the way the driving force was created in the two MD techniques. In VMD a vacuum pressure was applied to the permeate side, which created a very low permeate pressure. At the inlet of the membrane module the partial pressure of an aroma compound in the sweeping gas depends only on the molar fraction of the aroma compound and on the total pressure as both will remain almost constant after condensation. Along the membrane module the driving force obtained in SGMD depends upon the humidity of the sweeping gas and thus its temperature. The driving force obtained in SGMD was considerable lower than in VMD at the same experimental conditions (feed flow rate and temperature).

The MD flux is affected by the polarization effect. As a consequence the mass flux of component i (N_i) can be written as a function of the transmembrane partial pressure difference (ΔP_i) (Khayet et al., 2002).

$$N_i = B_i \Delta P_i \qquad (2)$$

Where B_i is the net MD coefficient of component i, B is dependent on membrane characteristics (thickness, tortuosity etc.) and parameters (temperature, pressure etc.). Saturation of the permeate through the membrane module will influence the mass flux of SGMD, since the permeate pressure will increase along the membrane module and thus reduce the transmembrane partial pressure difference. In fact, the partial pressure difference will be lower from the inlet to the outlet of the membrane module. Saturation of the permeate does not occur in VMD because of the very low permeate pressure. VMD and SGMD also differ with respect to mass transport through the membrane. In VMD the Knudsen diffusion mechanism dominates the mass transfer through the membrane because only traces of air can exist within the membrane pores (vacuum pressure) (Izquierdo-Gil & Jonsson, 2003). In SGMD the mass transport takes place via a combined Knudsen/molecular diffusion mechanism because air is present in the membrane pores (Khayet et al., 2000). As a consequence the net MD coefficient (B_i) is expressed differently for the two MD techniques:

$$VMD: B_i = K_m \sqrt{M_i} \tag{3}$$

$$SGMD: B_i = \frac{M_i}{RT}\left(\frac{1}{D_K} + \frac{P_{air}}{D_M}\right)^{-1}\frac{1}{\delta} \tag{4}$$

Where K_m is the permeability coefficient (depends on membrane geometry, structure and temperature), M_i is the molar mass of i, R is the gas constant, T is temperature, D_K is Knudsen diffusion coefficient, D_M is the molecular diffusion coefficient, P_{air} is the pressure of the air entrapped in the pores and δ is the membrane thickness (Lawson & Lloyd, 1997; Khayet et al., 2002; Izquierdo-Gil & Jonsson, 2003).

Concentration and temperature polarization are the main mass transfer resistances in the membrane-feed boundary layer in both MD techniques (Couffin et al., 1998; Garcia-Payo et al., 2002). In the membrane-gas boundary layer the mass transfer resistances will differ between the two techniques. In VMD the convective transport creates a pressure gradient at the interface, which results in a negligible resistance. In SGMD diffusion resistance and temperature polarization at the interface creates a mass transfer resistance, which is higher than the resistance in the feed boundary layer and thus dominant for the process.

Comparison of results of the SGMD technique with the VMD technique was difficult partly because the membrane module designs used were different resulting in different membrane areas, linear velocities and types of flow (Reynolds number) and partly because the rate of the sweeping gas flow rate used in the experimental set up was not optimal for the process, as saturation occurred.

4.2.1 Comparison of results: Model solution as feed

In both techniques an increase in the feed flow rate only had a minor effect on the flux (Table 2 and 4), whereas an increase in temperature had a considerable effect on the flux, which increased several times as the temperature was increased from 10°C to 45°C (Table 3 and 4). Equation 1 shows the association between the flux (J) and the activation energy (E_a) and in figure 8 the Arrhenius plot illustrates the differences in temperature dependence between the

permeate flux of VMD, the permeate flux of SGMD and the saturated water vapour pressure with $|E_{a,flux\text{-}SGMD}| > |E_{a,flux\text{-}VMD}| > |E_{a,water}|$. With Equation 2 in mind, it seems likely that the temperature dependency of the permeate flux in VMD is about 17% higher than the increase in the saturated water vapour pressure at increasing temperature: Besides the increase in driving force the coefficient B_i contains the diffusion coefficient which does also increase with increasing temperature. In the case of the permeate flux in SGMD the driving force is certainly lowered by the partial pressure of the water vapour on the permeate side due to the maximum water vapour uptake because of partly saturation of the sweeping gas. An increase in the feed temperature will cause the sweeping gas temperature to increase close to the feed temperature by heat and mass exchange why the gas can take up more water vapour before saturation occurs. This is reflected in an increase in the driving force compared to VMD giving a 19% higher temperature dependency than for the permeate flux in VMD. Thus the permeate flux obtained by VMD was approximately 10 times higher at 45°C and 16 times higher at 10°C (Table 3 and 4).

An increase in feed flow rate had a larger effect on the concentration factors obtained by VMD than by SGMD since an increase in feed flow rate mainly reduces concentration polarization, which is much more pronounced in VMD (Table 2 and 4). At the same experimental conditions: 30°C and a linear velocity of 0.8 m/s (VMD: 100 L/h, SGMD: 300 L/h) the CFs obtained by VMD were either significantly higher or at the same level as the ones obtained by SGMD (Table 2 and 4). This was because of the lower driving force in SGMD. With basically the same experimental driving force in the two MD techniques (VMD: 10°C, 400 L/h; SGMD: 45°C, 400 L/h) the CFs obtained by VMD were either significantly higher or at the same level as the ones obtained by SGMD (Table 3 and 4). This finding is due to the saturation of the sweeping gas where the aroma compounds had a higher degree of saturation than water. It was the highly volatile aroma compounds, which in both techniques obtained the highest concentrations factors: Ethyl butanoate, isoamyl acetate and 1,8 cineole (Table 3 and 4).

The linear correlation between the permeate flux and the water vapour pressure difference presented in figure 3 illustrates that the difference in water vapour pressure was the real driving force across the membrane in VMD with a net MD coefficient (B_{water}) of 0.007471 kg/(h m^2 Pa). The permeability coefficient (K_m) of VMD was equal to 1.546×10^{-5} (s mol^{½}/ m kg^{½}), which was in accordance with the value we previously obtained with black currant juice (Bagger-Jørgensen et al., 2004). The slopes of the correlations in figure 3 resemble the water permeability and the difference between VMD and SGMD is evident. The VMD water permeability is over 9 times the water permeability of SGMG (Figure 3). The relatively lower water permeability obtained with SGMD is mainly a result of the rather high average water vapour pressure obtained at the sweeping gas side, resulting in low driving forces and fluxes compared to VMD.

As judged from the data obtained with the model solution the VMD technique appears to be the best method to strip aroma compounds from black currant and cherry juice. However, the linear velocity at a given feed flow rate was 2.5 times higher in VMD than in SGMD resulting in correspondingly higher mass transfer coefficients and the rate of the sweeping gas flow was not optimal for the SGMD process, as saturation occurred. An increase in the linear velocity of the feed of SGMD may have a limited influence on the concentration factors of the aroma compounds whereas an increase in the sweeping gas flow rate most likely would increase the permeate flux. The influence on the concentration factors, however, would depend upon the difference in the degree of saturation of the aroma compounds and of the water in the sweeping gas. The SGMD flux obtained at 45°C would at a higher sweeping gas flow rate be significantly

higher than the VMD flux obtained at 10°C. However, even at a low degree of saturation of water compared to the aroma compounds in the sweeping gas the concentration factors of SGMD would probably still be significantly lower than the concentrations factors of VMD.

4.2.2 Comparison of results: Black currant juice as feed

In the black currant juice experiments the only parameter examined was the temperature. An increase in the temperature had, in both MD techniques, a significant effect on the permeate flux and on the CFs obtained except for 4-terpinenol (VMD) and cis-3-Hexen-1-ol (SGMD) (Table 5). The insignificant influence of the temperature on the level of the two compounds was also seen with the model solution. The VMD and SGMD fluxes were identical for the black currant juice and the model solution. The CFs of the five selected aroma com-pounds were, in both MD techniques, either at the same level or lower than the ones obtained with the model solution (Table 3, 4 and 5). An explanation could be that a microenvironment exists within the black currant juice, which mainly reduces the volatility of the highly volatile aroma compounds. When black currant juice is produced some of the seeds are crushed releasing aromatic oils for which the affiliation of the hydrophobic aroma compounds is higher, reducing their volatility. The aroma compounds could also bind to remains of cell wall material like glucosides and pectin, which would reduce their volatility. In both MD techniques the highly volatile aroma compounds: Ethyl butanoate, isoamyl acetate and 1,8 cineole had significantly higher CFs than the poorly volatile aroma compounds: cis-3-Hexen-1-ol and 4-terpinenol, which is in accordance with the model solution experiments (Table 3, 4 and 5).

The fraction of recovered aroma as a function of feed volume reduction can be calculated by Equation (5), where K is the concentration factor, V is the molar volume and C is the concentration (Bagger-Jørgensen et al., 2004). The CFs of the aroma compounds were assumed to be constant.

$$\frac{V_{retentate} \times C_{retentate}}{V_{feed} \times C_{feed}} = \left(\frac{V_{retentate}}{V_{feed}} \right)^{K} \quad \text{with} \quad K = \frac{C_{permeate}}{C_{feed}} \qquad (5)$$

In figure 4 (SGMD) and 5 (VMD) the theoretically recovered aroma fraction $(1-(V_{retentate}/V_{feed})^{K})$ as a function of the feed volume reduction $(1-(V_{retentate}/V_{feed}))$ is compared with the experimental data from the black currant experiments. The grouping of the 5 aroma compounds in high and poor volatility is evident in the figures, with small individual differences within the groups between the two MD techniques. The experimental and predicted values of the highly volatile compounds were, in both MD techniques, in accordance. The poorly volatile aroma compounds were in less accordance with the predicted values (Figure 4 and 5). It was evident that at the same flux level VMD requires a smaller volume reduction than SGMD to obtain a certain degree of recovered aroma of all five aroma compounds (Figure 4 and 5). The main reason for this difference is the saturation of the sweeping gas in SGMD.

5. Conclusions

Within the experimental parameters examined the feed temperature had by far the largest effect on the SGMD flux, which was increased almost exponentially due to an increase in the partial pressure difference. An increase in the feed flow rate and sweeping gas flow rate also increased

the permeate flux because of a decrease in the temperature polarization and in the saturation degree of the permeate, respectively. The experimental data showed that an increase in temperature and feed flow rate increased the concentration factors of most of the aroma compounds examined. The increase in temperature increased the aroma flux more than the water flux thus resulting in a higher permeate concentration. Oppositely, an increase in the feed flow rate reduces the concentration polarization thus resulting in a higher aroma concentration at the membrane surface and consequently a higher permeate concentration. A change in temperature had by far the largest effect. At 45°C (400 L/h) the highly volatile aroma compounds (hydrophobic) obtained the highest concentration factors: 12.1–9.3 (black currant juice), 17.2-12.8 (model solution). Within the experimental parameters examined the sweeping gas flow rate did not have influence on the concentration factors.

Compared with VMD the SGMD technique works with a substantially higher permeate pressure resulting in lower driving force and consequently a lower water permeability. The influence of the feed flow rate is less in SGMD since concentration polarization is more pronounced in VMD. SGMD is, however, more influenced by an increased in feed temperature than VMD because the temperature of the sweeping gas and thus the amount of vapour the sweeping gas can contain before saturation occurs will increase.

With a black currant juice volume reduction of 13.7 vol.% (45°C, 400 L/h) the amount of recovered aroma with SGMD was 73-84 vol.% for the highly volatile compounds. From a differential mass balance over the MD process the theoretically predicted aroma recovery as a function of the feed volume reduction was calculated. The experimental values of especially the highly volatile compounds were in very good accordance with the predicted values. To obtain a certain degree of recovered aroma SGMD required a significant larger volume reduction than VMD due to the lower driving force and further concentration of the SGMD permeate is necessary.

The experimental results imply that SGMD has potential as a technique for gentle recovery of thermally sensitive fruit juice aroma, perhaps as part of an integrated process. There exist other MD techniques besides SGMD and VMD and the applicability of osmotic membrane distillation and direct contact membrane distillation for aroma recovery during juice concentration deserves examination.

Nomenclature

B	net membrane distillation coefficient	(-)
C	concentration	(kg / m^3)
D_K	Knudsen diffusion coefficient	(m^2 / s)
D_M	molecular diffusion coefficient	(Pa m^2 / s)
E_a	activation energy	(J / mol)
J_o	molar flux at set point temperature	(mol / m^2 s)
J	molar flux	(mol / m^2 s)
K	concentration factor	(-)
K_m	permeability coefficient	(-)
M	molar mass	(kg / mol)
N	mass flux	(kg / s m^2)
P	pressure	(Pa)
$\Delta P_i^{'}$	partial pressure gradient	(Pa)
R	gas constant	(m^3 Pa / mol K)
T	temperature	(K)
V	molar volume	(m^3 / mol)

Subscripts

f	feed
i	the component
mf	membrane-feed interface
mp	membrane-permeate interface
p	permeate

Greek letters

δ	membrane thickness	(m)

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Figure captions

Figure 1. The 12 aroma compounds selected for examination in this study.

Figure 2. Schematic representation of sweeping gas membrane distillation apparatus.
1) Membrane module, 2) Feed reservoir, 3) Feed pump, 4) Thermostat,
5) a-b Flow meters, 6) a-d Manometers, 7) a-d Thermometers, 8) Condensation trap, with cooled ethanol, 9) Gas pump, 10) a-c Valves.

Figure 3. The permeability of the membrane depicted as flux (of juice) vs. the water vapour pressure difference between the feed and permeate side of the membrane (P_{feed} - $P_{permeate}$).

Figure 4. The fractions of aroma recovered by sweeping gas membrane distillation shown with the standard deviation (45°C, 400 L/h, 2 m^3/h) versus the theoretically calculated values of aroma recoveries (Eq. 5, the concentration factors (*K*) are from table 5 (45°C)).

Figure 5. The fractions of aroma recovered by vacuum membrane distillation shown with the standard deviation (10°C, 400 L/h) versus the theoretically calculated values of aroma recoveries (Eq. 5, the concentration factors (*K*) are from table 5 (10°C)).

Figure 6. Theoretically assumed profiles of transmembrane concentration, temperature and partial pressure gradients in SGMD. The solid lines illustrate the profiles at low feed flow rate and low temperature, while the dotted lines illustrate the profiles at either high feed flow rate (T and C) or high temperature (P) (not to scale). (Rivier, Garcia-Payo, Marison & von Stockar, 2002).

Figure 7. Theoretically assumed profiles of concentration and temperature gradients along the membrane (the feed flow direction) in SGMD. The solid lines illustrate the feed (f) or permeate (p) profiles, while the dotted lines illustrate the profiles at the two membrane surfaces (mf, mp) (not to scale). The arrows indicate the countercurrent between the sweeping gas and feed flow (Khayet et al., 2000; Rivier et al., 2002).

Figure 8. Temperature dependence of the saturated pressure (P^{sat}) of water, 3-methyl-1-butanol and ethyl butanoate (solid lines) shown together with the temperature dependence of the permeate flux (l/h/m^2) of SGMD (flux-SGMD) and of VMD (flux-VMD) (dotted lines).

Table 1

Physical data[1] of the aroma compounds

Aroma compound[2]	Molecular formula	Molecular weight (g/mol)	Retention time GC-MS (min.)	Boiling point (Kelvin)	Aqueous solubility at pH 3 (g/L)	LogP
Diacetyl	$C_4H_6O_2$	86.1	3.85	362.8	1000	-1.33 ± 0.30
Furfural	$C_5H_4O_2$	96.1	21	436.8	19.15	0.73 ± 0.26
3-methyl-1-butanol	$C_5H_{12}O$	88.1	13.78	405.9	29.54	1.22 ± 0.19
cis-3-Hexen-1-ol	$C_6H_{12}O$	100.2	19.85	431.2	14.89	1.61 ± 0.20
Ethyl butanoate	$C_6H_{12}O_2$	116.2	5.87	397.3	5.75	1.77 ± 0.21
Benzaldehyde	C_7H_6O	106.1	23.09	453.9	2.41	1.64 ± 0.24
Isoamyl acetate	$C_7H_{14}O_2$	130.2	8.92	417.1	2.54	2.12 ± 0.21
Eugenol	$C_{10}H_{12}O_2$	164.2	35.76	530.3	1.87	2.20 ± 0.24
1,8-Cineole	$C_{10}H_{18}O$	154.3	13.16	449.2	0.45	2.82 ± 0.27
Linalool	$C_{10}H_{18}O$	154.3	23.77	473.4	0.67	3.28 ± 0.26
4-Terpinenol	$C_{10}H_{18}O$	154.3	25.20	483.9	2.10	2.99 ± 0.24

[1] Calculated with ACD/Labs software version 7.0

[2] It was not possible to calculate the physical data of the compound β-Damascenone ($C_{13}H_{18}O$, 190.1 g/mol, Ret. Time: 29.72)

Table 2

SGMD[1] (model solution): The concentration factors from the feed flow rate experiments[2] shown with their standard deviation

Flow rate (L/h)	Permeate flux (L/h/m^2)	Diacetyl	Ethyl butanoate	Isoamyl acetate	1,8-Cineole	3-methyl-1-butanol	*cis*-3-Hexen-1-ol	Furfural	Benzaldehyde	Linalool	4-Terpinenol	β-Damascenone	Eugenol
300	1.93^{a}	5.7±0.6^{a}	6.6±0.5^{a}	6.6±0.5^{a}	9.6±0.1^{a}	6.0±0.3^{a}	2.6±0.3^{a}	4.5±0.3^{a}	4.2±0.1^{a}	7.9±0.8^{a}	5.5±0.4^{a}	6.4±0.4^{a}	4.5±0.4^{a}
400	1.99^{ab}	6.1±0.5^{a}	7.6±0.4^{ab}	7.6±0.6^{ab}	11.5±0.8^{ab}	7.0±0.3^{a}	2.9±0.2^{a}	4.9±0.1^{a}	4.9±0.4^{a}	8.5±0.3^{a}	5.4±0.5^{a}	7.2±0.6^{a}	4.5±0.7^{a}
500	2.04^{b}	6.0±0.1^{a}	9.1±0.8^{b}	9.4±0.9^{b}	12.5±1.2^{b}	7.1±1.0^{a}	3.2±0.6^{a}	5.2±0.3^{a}	5.6±0.8^{a}	9.5±0.6^{a}	6.0±0.7^{a}	8.0±1.1^{a}	5.6±0.5^{a}

[1] Constant sweeping gas flow rate: 2m^3/h

[2] Constant inlet temperature: 30^{o}C

Superscript letters a and b indicates groupings within a column. Different letters show statistical difference $p<0.05$.

Table 3

SGMD (model solution): The concentration factors from the temperature experiments[1] shown with their standard deviation

Sweeping gas flow rate (m^3/h)	Temperature (°C)	Permeate flux (L/h/m^2)	Diacetyl	Ethyl butanoate	Isoamyl acetate	1,8-Cineole	3-methyl-1-butanol	*cis*-3-Hexen-1-ol	Furfural	Benzaldehyde	Linalool	4-Terpinenol	β-Damascenone	Eugenol
2	10	0.27^{a}	1.9 ± 0.1^{a}	1.6 ± 0.1^{a}	1.6 ± 0.3^{a}	2.1 ± 0.1^{a}	2.4 ± 0.4^{a}	1.2 ± 0.1^{a}	2.7 ± 0.1^{a}	1.3 ± 0.1^{a}	2.9 ± 0.4^{a}	2.5 ± 0.1^{a}	2.2 ± 0.1^{a}	3.6 ± 0.5^{a}
2	20	0.89^{b}	4.2 ± 0.5^{b}	3.7 ± 0.5^{b}	3.6 ± 0.6^{a}	5.6 ± 1.1^{b}	4.4 ± 0.6^{a}	1.9 ± 0.2^{a}	3.6 ± 0.4^{ab}	2.7 ± 0.4^{b}	5.4 ± 1.2^{a}	4.0 ± 0.7^{ab}	4.3 ± 1.3^{ab}	4.0 ± 0.1^{a}
2	30	1.99^{c}	6.1 ± 0.5^{c}	7.6 ± 0.4^{c}	7.6 ± 0.6^{b}	11.5 ± 0.8^{c}	7.0 ± 0.3^{b}	2.9 ± 0.2^{ab}	4.9 ± 0.1^{b}	4.9 ± 0.4^{c}	8.5 ± 0.3^{b}	5.4 ± 0.5^{b}	7.2 ± 0.6^{bc}	4.5 ± 0.7^{a}
2	40	3.88^{d}	6.3 ± 0.1^{c}	11.4 ± 0.4^{d}	11.6 ± 0.7^{c}	13.4 ± 0.1^{d}	7.7 ± 0.1^{bc}	3.3 ± 0.2^{b}	5.5 ± 0.3^{bc}	6.5 ± 0.2^{d}	9.9 ± 1.3^{bc}	6.6 ± 0.9^{bc}	7.8 ± 1.4^{c}	5.0 ± 0.9^{a}
2	45	5.04^{e}	8.4 ± 0.7^{d}	13.0 ± 0.3^{e}	13.1 ± 1.1^{c}	16.3 ± 0.5^{e}	9.6 ± 1.2^{cd}	3.6 ± 0.5^{b}	6.9 ± 1.1^{c}	7.4 ± 0.6^{d}	11.8 ± 1.4^{c}	8.7 ± 1.5^{c}	9.5 ± 0.6^{c}	5.9 ± 1.1^{a}
1.2	45	4.01^{d}	9.5 ± 0.5^{d}	13.0 ± 0.7^{e}	12.8 ± 1.3^{c}	17.2 ± 0.6^{e}	9.8 ± 0.6^{d}	3.8 ± 0.5^{b}	7.0 ± 0.6^{c}	7.7 ± 0.8^{d}	10.6 ± 0.1^{bc}	8.3 ± 0.1^{c}	9.7 ± 1.2^{c}	5.3 ± 0.5^{a}

[1] Constant feed flow rate: 400 L/h

Superscript letters a, b, c, d and e indicates groupings within a column. Different letters show statistical difference $p<0.05$.

Table 4
VMD (model solution): The concentration factors from the temperature[1] and feed flow rate[2] experiments shown with their standard deviation

Parameter (T= temperature, F= Feed flow rate)	Permeate flux ($L/h/m^2$)	Diacetyl	Ethyl butanoate	Isoamyl acetate	1,8-Cineole	3-methyl-1-butanol	*cis*-3-Hexen-1-ol	Furfural	Benzaldehyde	Linalool	4-Terpinenol	β-Damascenone	Eugenol
T^1=10 ^{0}C	4.34^a	13.8±1.2^a	46.1±2.8^a	47.0±2.8^a	34.7±2.1^a	13.5±0.3^a	9.7±0.2^a	9.1±0.3^a	28.4±0.5^a	15.1±0.5^a	7.6±0.5^a	17.6±1.5^a	9.0±1.4^a
T^1=30 ^{0}C	20.99^b	9.7±1.7^b	18.6±0.2^b	19.6±0.9^b	16.1±0.3^b	10.9±0.4^b	5.9±0.4^b	7.5±0.2^b	13.8±0.2^b	11.4±0.4^b	7.7±0.7^a	11.3±1.6^b	7.0±0.9^{ab}
T^1=45 ^{0}C	48.55^c	8.8±1.3^b	13.7±1.2^c	14.5±1.3^c	12.4±0.8^c	9.6±0.4^c	5.4±0.5^b	7.4±0.6^b	11.2±0.7^c	9.4±0.6^c	7.6±0.2^a	9.7±0.9^b	5.2±0.5^b
F^2=100 L/h	17.86^a	5.4±0.5^a	9.8±0.2^a	9.5±0.3^a	9.4±0.7^a	6.7±0.3^a	5.4±0.1^a	5.8±0.2^a	8.8±0.2^a	6.3±0.3^a	5.2±0.1^a	6.4±0.2^a	6.3±0.7^a
F^2=300 L/h	19.16^b	6.2±1.1^a	16.4±0.8^b	16.2±2.1^b	15.5±1.2^b	9.9±0.7^b	5.4±0.6^a	7.4±0.3^b	13.3±0.2^b	9.8±0.1^b	7.6±0.4^b	10.8±0.4^b	6.8±1.6^a
F^2=400 L/h	20.99^c	9.7±1.7^b	18.6±0.2^c	19.6±0.9^b	16.1±0.3^b	10.9±0.4^b	5.9±0.4^{ab}	7.5±0.2^b	13.8±0.2^b	11.4±0.4^c	7.7±0.7^b	11.3±1.6^b	7.0±0.9^a
F^2=500 L/h	21.00^c	10.6±1.4^b	24.8±0.8^d	25.1±1.1^c	20.7±2.3^c	12.9±0.9^c	6.9±0.3^b	9.0±0.7^c	17.2±0.7^c	13.9±1.0^d	9.1±0.9^b	14.9±1.2^c	7.3±1.5^a

[1] Constant feed flow rate: 400 L/h
[2] Constant inlet temperature: 30^{o}C
Superscript letters a, b, c and d indicates grouping within a column (temperature or feed flow rate experiments). Different letters show statistical difference $p<0.05$.

Table 5
SGMD[1] and VMD (black currant juice): Concentration factors shown with their standard deviation

Feed[2]			Aroma compound				
Membrane distillation technique	Temperature (°C)	Permeate flux (L/h/m^2)	Ethyl butanoate	Isoamyl acetate	1,8-Cineole	*cis*-3-Hexen-1-ol	4-Terpinenol
SGMD	30	1.96	8.4 ±0.4 ^{a,y}	7.9 ±0.3 ^{a,y}	10.6 ±0.5 ^{a,z}	3.7 ±0.4 ^{a,x}	4.2 ±0.1 ^{a,x}
SGMD	45	4.91	10.3 ±0.5 ^{b,y}	9.3 ±0.5 ^{b,y}	12.1 ±0.4 ^{b,z}	4.0 ±0.2 ^{a,v}	5.5 ±0.3 ^{b,x}
VMD	10	4.21	27.4 ±3.2 ^{a,zy}	31.3 ±3.1 ^{a,z}	23.6 ±2.7 ^{a,y}	10.1 ±0.5 ^{a,x}	7.4 ±0.2 ^{a,x}
VMD	30	20.73	15.5 ±0.6 ^{b,y}	16.7 ±0.8 ^{b,z}	12.9 ±0.3 ^{b,x}	6.3 ±0.4 ^{b,v}	7.2 ±0.4 ^{a,v}

[1] Constant sweeping gas flow rate: 2m^3/h
[2] Constant feed flow rate: 400 L/h
Superscript letters a and b indicates grouping within a column (VMD or SGMD). Different letters show statistical difference $p<0.05$.
Superscript letters z, y, x and v indicates grouping within a row. Different letters show statistical difference $p<0.05$.

Figure 1

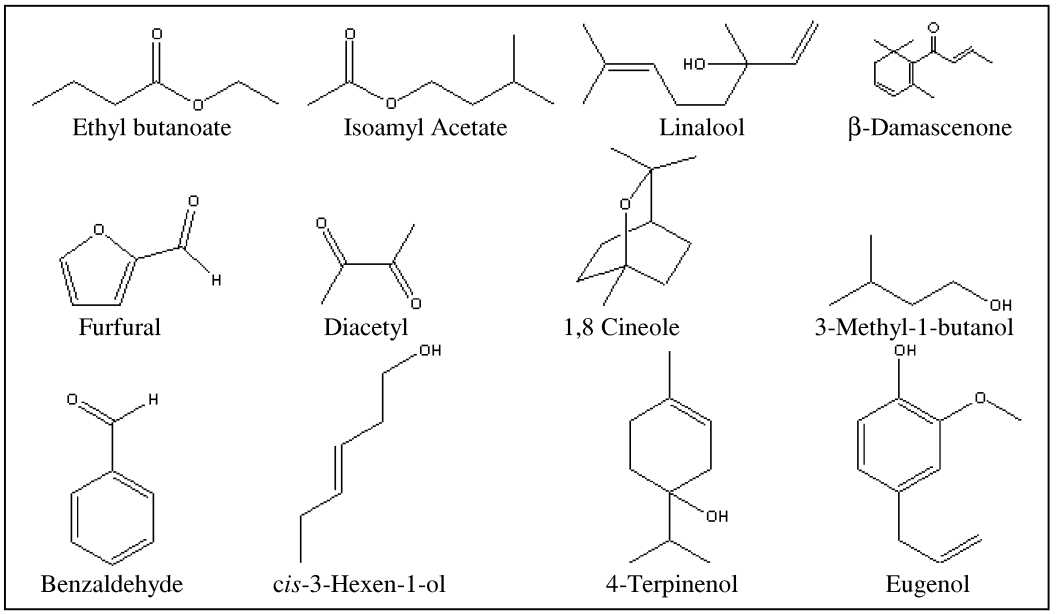

Ethyl butanoate
Isoamyl Acetate
Linalool
β-Damascenone
Furfural
Diacetyl
1,8 Cineole
3-Methyl-1-butanol
Benzaldehyde
cis-3-Hexen-1-ol
4-Terpinenol
Eugenol

Figure 2

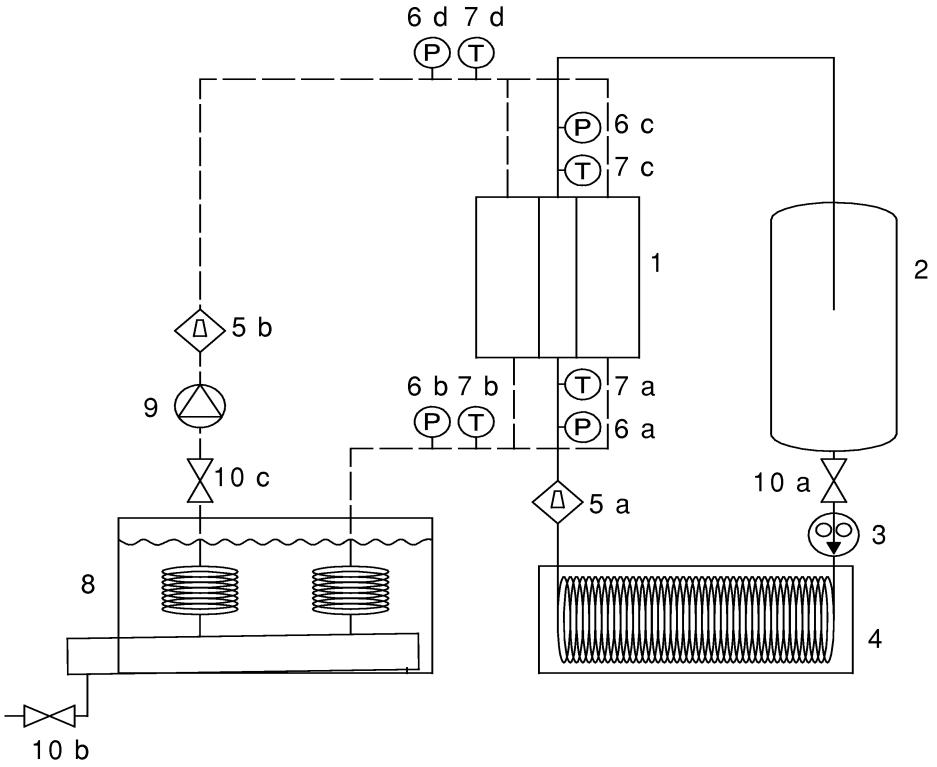


Figure 3

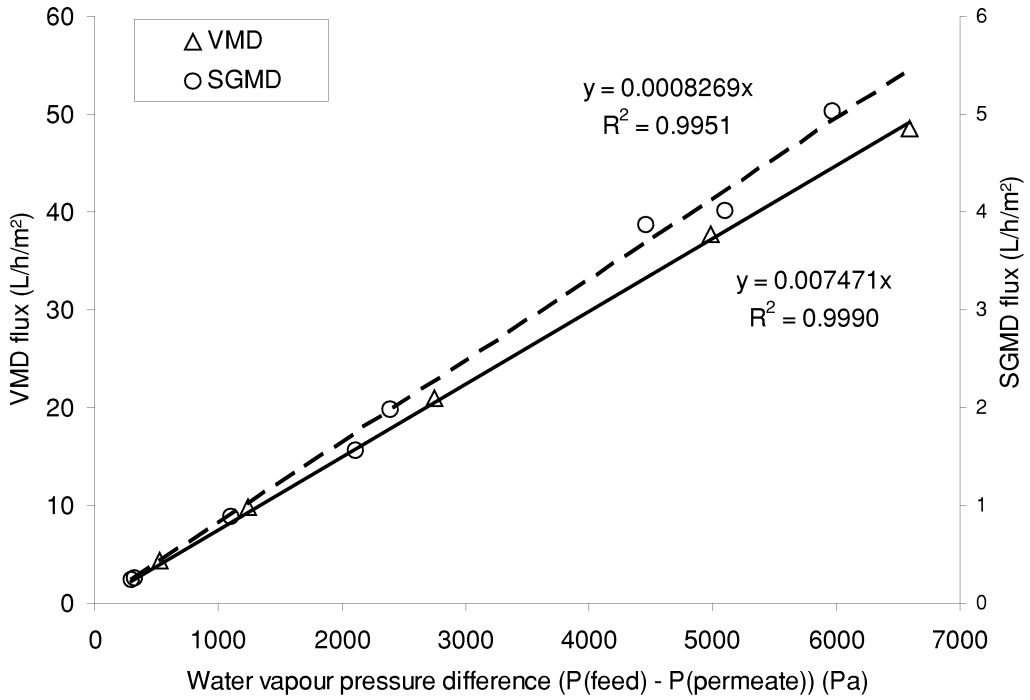

VMD
SGMD
y = 0.0008269x
R2 = 0.9951
y = 0.007471x
R2 = 0.9990
VMD flux (L/h/m2)
SGMD flux (L/h/m2)
Water vapour pressure difference (P(feed) - P(permeate)) (Pa)
0
10
20
30
40
50
60
0
1
2
3
4
5
6
0
1000
2000
3000
4000
5000
6000
7000

Figure 4

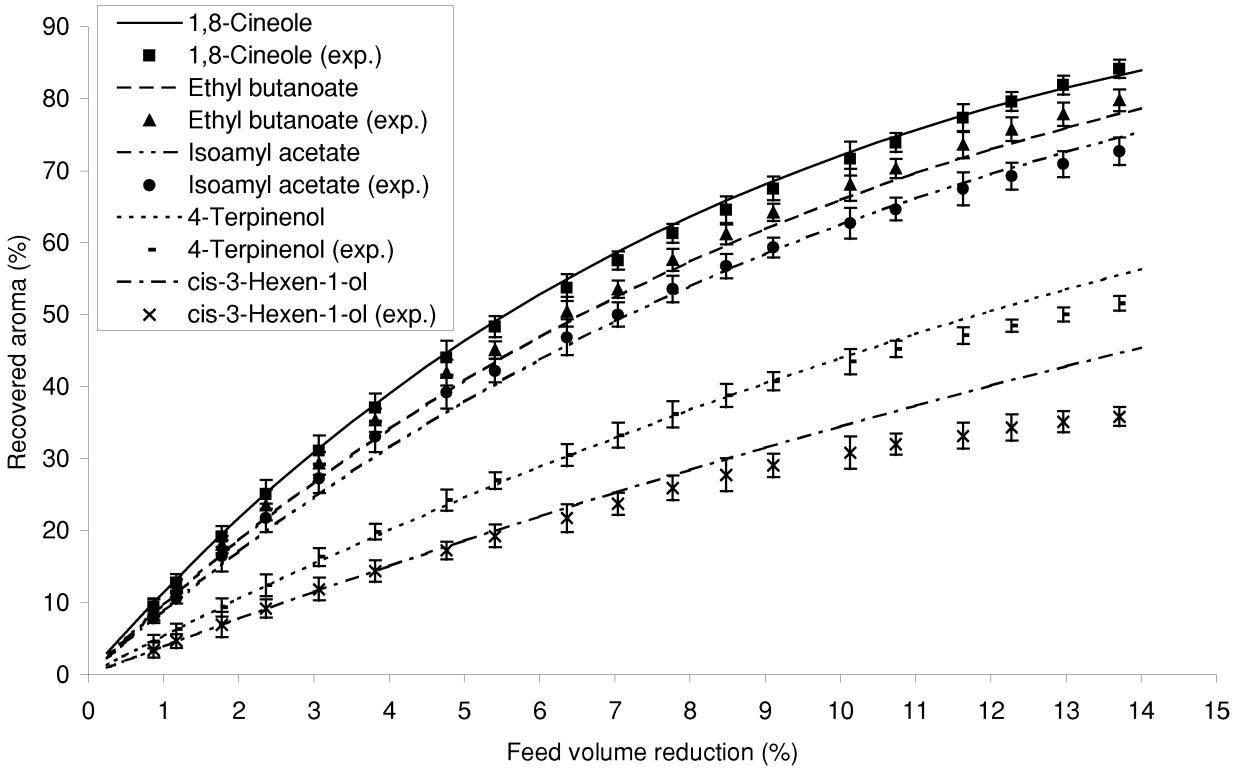

1,8-Cineole
1,8-Cineole (exp.)
Ethyl butanoate
Ethyl butanoate (exp.)
Isoamyl acetate
Isoamyl acetate (exp.)
4-Terpinenol
4-Terpinenol (exp.)
cis-3-Hexen-1-ol
cis-3-Hexen-1-ol (exp.)
Recovered aroma (%)
Feed volume reduction (%)
0
10
20
30
40
50
60
70
80
90
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15

Figure 5

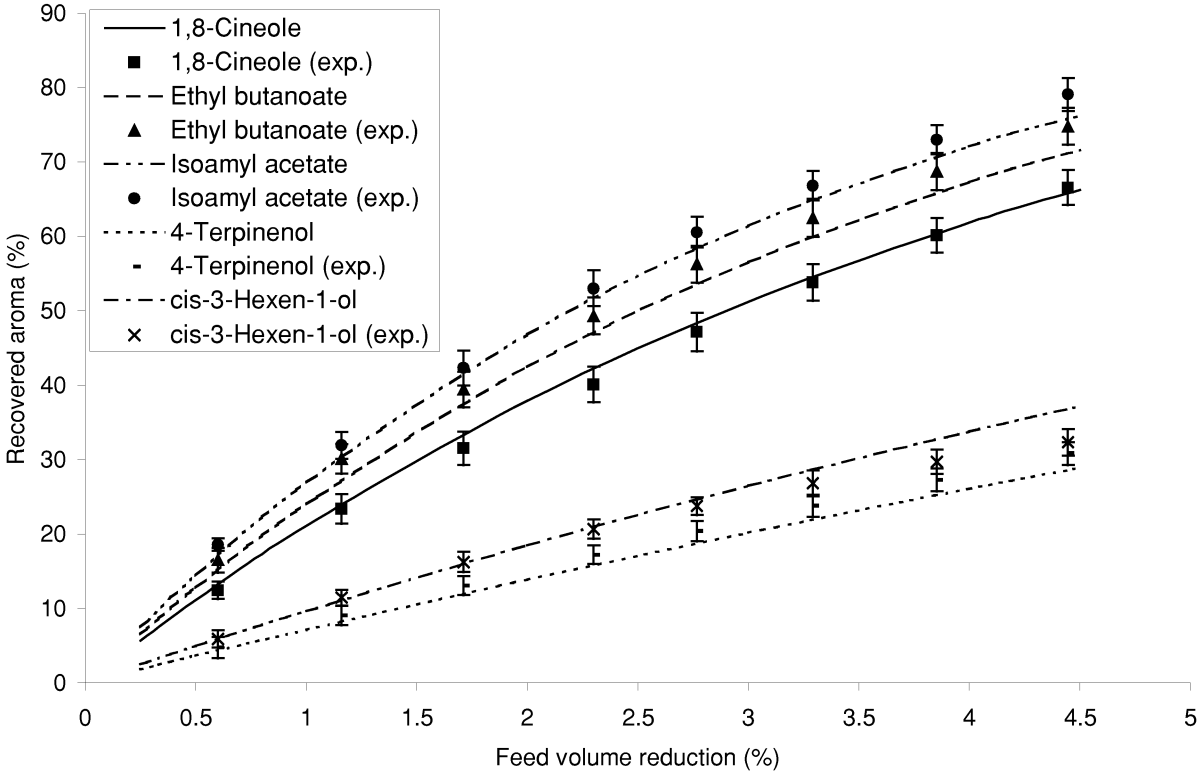

1,8-Cineole
1,8-Cineole (exp.)
Ethyl butanoate
Ethyl butanoate (exp.)
Isoamyl acetate
Isoamyl acetate (exp.)
4-Terpinenol
4-Terpinenol (exp.)
cis-3-Hexen-1-ol
cis-3-Hexen-1-ol (exp.)
Recovered aroma (%)
Feed volume reduction (%)
0
10
20
30
40
50
60
70
80
90
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5

Figure 6

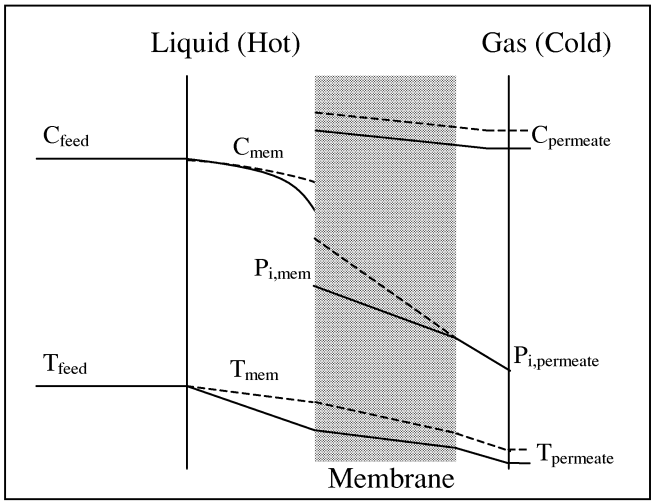

Liquid (Hot)
Gas (Cold)
C_{feed}
C_{mem}
$C_{permeate}$
$P_{i,mem}$
$P_{i,permeate}$
T_{feed}
T_{mem}
$T_{permeate}$
Membrane

Figure 7

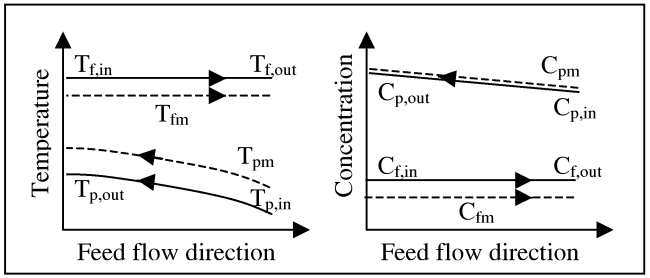
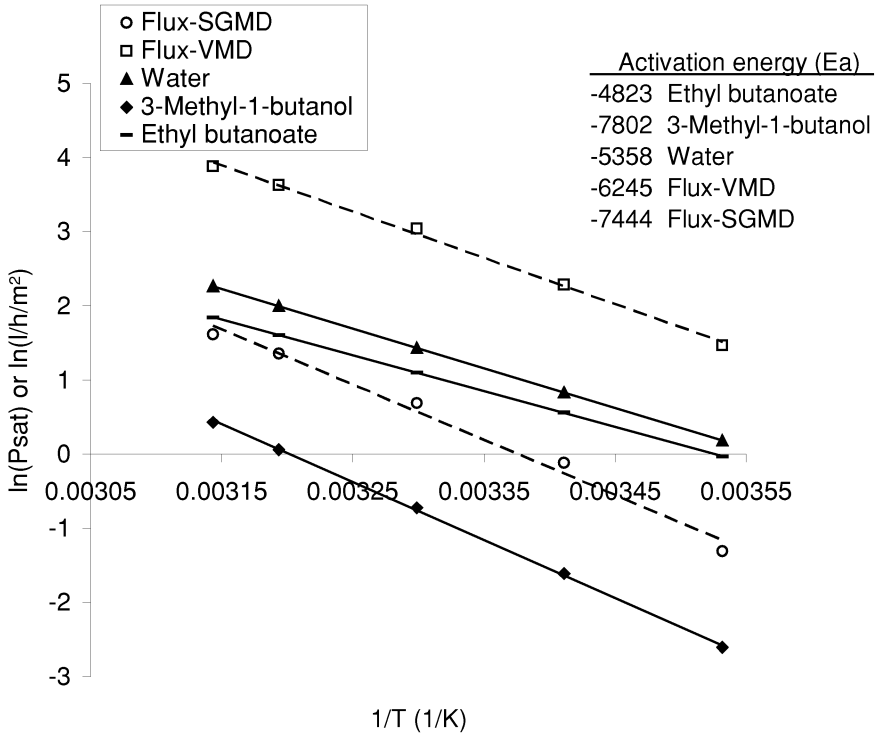

Temperature
$T_{f,in}$
$T_{f,out}$
T_{fm}
T_{pm}
$T_{p,out}$
$T_{p,in}$
Feed flow direction
Concentration
C_{pm}
$C_{p,out}$
$C_{p,in}$
$C_{f,in}$
$C_{f,out}$
C_{fm}
Feed flow direction

Figure 8


Flux-SGMD
Flux-VMD
Water
3-Methyl-1-butanol
Ethyl butanoate
Activation energy (Ea)
-4823 Ethyl butanoate
-7802 3-Methyl-1-butanol
-5358 Water
-6245 Flux-VMD
-7444 Flux-SGMD
ln(Psat) or ln(l/h/m²)
1/T (1/K)
5
4
3
2
1
0
-1
-2
-3
0.00305
0.00315
0.00325
0.00335
0.00345
0.00355

Paper VII

Monoterpenes in black currant (*Ribes nigrum* L.) juice: Role of heat induced changes and glycosidically bound terpenes

Submitted to *Journal of Agricultural and Food Chemistry.*

Varming, C., Andersen, M. L. and Poll, L.

Monoterpenes in Black Currant (*Ribes nigrum* L.) Juice: Role of Heat Induced Changes and Glycosidically Bound Terpenes

Camilla Varming[*)], Mogens L. Andersen, and Leif Poll

Department of Food Science, Royal Veterinary and Agricultural University, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark

ABSTRACT

Limonene, α-terpinene, linalool, α-terpineol, 4-terpineol and menthol added to either black currant juice or a model system were thermally treated at 90 °C for 30 minutes. Similar heat induced decreases in concentration of the terpenes were observed in the two systems. A range of terpene hydrocarbons and oxygenated terpenes were formed, α-terpineol being a main conversion product of most of the examined compounds. The loss of compounds generally exceeded the concentrations of products formed. In addition, determination of glycosidically bound terpenes in the juice was carried out by two methods of enzymatic hydrolysis, namely addition of β-glucosidase to an Amberlite XAD-2 isolate or directly to black currant juice. The two methods gave the same patterns of seven released volatile aglyconic terpenes. However, none of the released terpenes are important for the aroma of black currant juice.

Keywords: black currant juice, (*Ribes nigrum* L.), thermal treatment, terpenes, aroma, glycosidically bound, acid catalysis

*) Tel.: (+45)35283435, Fax.: (+45)35283265, E-mail address: cva@kvl.dk

INTRODUCTION

The industrial production of black currant berries to juice involves enzyme treatment, pressing, clarification, filtration and several heating steps. Heat facilitated concentration of fruit juices is in addition often applied in order to reduce volume and stabilize the juice for storage and transport. Each processing step alters to some extent the aroma profile of the berry and especially operations involving the application of heat can lead to major changes in aroma and sensory characteristics (*1-7*).

Terpenes together with esters and alcohols are the major groups of aroma compounds in black currant juice. Some of the volatile terpenes have been identified by gas chromatography-olfactometry (GC-O) as being important for black currant juice aroma (*1,8,9*). Terpenes are converted to other terpenes via the hydration of double bonds, dehydration, rearrangements and cyclisation under acidic conditions similar to those found in black currant juice (*10*). Early work by von Sydow and co-workers showed that large changes in the monoterpene complex occurs when black currant juice or mash is subject to heat-treatment (*11*), and the sensory changes that take place during heating can be correlated with the decrease of terpene hydrocarbons and an increase of other compounds (*12*). More recently, thermal treatment of black currant juice has been shown to cause an increase in the concentration of several terpenes, such as limonene, α-terpinene and α-terpineol, and a decrease in the concentration of a few terpenes, namely linalool and 4-terpineol (*7*). A loss of most terpenes occurs during the concentration of black currant juice, whereas some oxygenated terpenes are recovered in larger amounts than in the base juice, suggesting that these compounds are released from a pool of glycosides or otherwise bound species (*5,6,13*). Glycosidically bound aroma compounds can be released from the precursors in fruits by the action of e.g. heat, acid hydrolysis, or enzymes. Hence, the changes in terpene concentrations during thermal processing might be a combination of acid-catalyzed terpene reactions, and release from a pool of glycosidically bound terpenes. The presence of monoterpene glycosides in black currants has previously only been indicated (*14*), but recently a range of aliphatic and aromatic alcohols were identified as glycosidically bound in black currant juice (*15*).

The aim of the present study was to evaluate the importance of acid catalyzed reactions and release of glycosidically bound compounds as the causes of the changes observed in the terpene complex during thermal treatment of black currant juice. Thermal treatments of single terpene aroma compounds were performed in juice, as well as in model solutions in order to detect possible concomitant terpene formations and degradations. The role of glycosidically bound terpenes was studied by examining the effect of enzymatic hydrolysis by application of β-glucosidase in juice and on isolated glycosides.

MATERIALS AND METHODS

Materials. A commercial black currant juice of the variety Ben Lemond was obtained from an industrial plant. Details of the juice preparation is described in (*7*). The juice was stored at -18°C and thawed immediately before use.

Model solution resembling black currant juice (glucose conc. 48g/L, fructose conc. 61g/L, citric acid monohydrate conc. 39 g/L in water, 14 °Brix) and single terpene (limonene, α-terpinene, linalool, α-terpineol, 4-terpineol or menthol) in model solutions (0.5 mg/L) adjusted to pH 3.1 (same as black currant juice). 0.1M citrate-phosphate buffer (pH 5). Almond ß-glucosidase enzyme solution (10 mg/mL, Sigma-Aldrich, Denmark) in 0.1M citrate-phosphate

buffer (pH 5). Amberlite XAD-2 (20-60 mesh) adsorbent resin (Sigma-Aldrich, Copenhagen, Denmark). The aroma standards used were obtained as follows: α–terpinene, linalool oxide, rose oxide, cumin aldehyde, and *p*-cymene (Fluka, Buchs, Switzerland); menthol, menthone, citronellol, bornyl acetate, α-terpinolene and 4-terpineol (Roth, Karlsruhe, Germany); β-damascenone (Firmenich, La Plaine, Switzerland), and the remaining compounds were from (Sigma-Aldrich, Copenhagen, Denmark).

Heating of Samples. One hundred millilitres of black currant juice or single terpene model solution (0.5 mg/L) was transferred into a 250 mL blue cap flask equipped with a screw cap. Heating was performed in a closed system in order to avoid evaporation. To obtain the desired temperature of 90 °C samples were heated in a microwave oven (Samsung Classic Collection microwave oven-b30^{TC}) and then transferred to a preheated 90 °C water bath, where kept under magnetic stirring for 30 min. Immediately after heating, the samples were cooled in an ice-water bath and stored at 5 °C until solvent extraction was carried out. Control samples were not subject to any heating. The heat treatments were performed in triplicates.

Solvent Extraction of Aroma Compounds. Fifty milliliters of black currant juice or single terpene model solution was transferred into a 250 mL blue cap flask and added 50 mL of diethyl ether/pentane 1:1 and 1,00 mL internal standard (50 µL/L 4-methyl-1-pentanol, Aldrich, Steinheim, Germany). Volatiles were extracted for 30 min under magnetic stirring (100 rpm). The sample was then left for separation of phases for 15 min, and placed in a freezer allowing the water phase to freeze and the solvent phase to be decanted. The solvent phase was then dried with Na_2SO_4 and concentrated to 0.080 g under a gentle stream of nitrogen. The extract was stored at -18°C until GC analysis. **Quantification.** Quantifications for the solvent extraction method were carried out with terpene standards in model solutions (0.5 mg/L). Extractions were performed in duplicate.

Glycoside Isolation. Isolation of glycosidically bound volatiles was performed according to the method described by Boulanger (*16*). One hundred milliliters of black currant juice was poured (1.5 mL/min) onto a solvent washed XAD-2 column (9*1.6 cm) and water soluble components were eluted with 50 mL of distilled water. The free volatile fraction was extracted with 50 mL pentane/diethylether (1:1) (1.5 mL/min) and discharged. The glycosidically bound aroma fraction was extracted with 50 mL methanol (1.5 mL/min), concentrated under reduced pressure to approx. 1 ml and redissolved in 2.0 mL 0.1M citrate-phosphate buffer (pH 5). The glycosidic extract was washed twice with 2 mL of pentane/diethylether (1:1).

Enzymatic Hydrolysis. Enzymatic hydrolysis of chromatographically separated glycosidic extract: Half of the glycosidic extract was added 2.0 mL of β-glucosidase in 0.1M citrate-phosphate buffer (pH 5) and the other half was added 2.0 mL of 0.1M citrate-phosphate buffer (pH 5), constituting the control sample.

Direct enzymatic hydrolysis: Black currant juice and 0.1M citrate-phosphate buffer (pH 5) (1:1) was adjusted to pH 5. Fifty milliliter of the mixture was transferred to a 250 mL glass flask equipped with a lid and 2.0 ml of ß-glucosidase in 0.1M citrate-phosphate buffer (pH 5) was added. The juice control sample constituted 50 mL of the juice and buffer (1:1) and 2.0 mL of 0.1M citrate-phosphate buffer (pH 5).

All samples were incubated for 19h in a 37°C water bath.

Dynamic Headspace Collection. Immediately after the enzyme treatments dynamic headspace collection was carried out. The chromatographically separated glycosidic extract sample was transferred to a 250 mL glass flask equipped with a purge head and dissolved with

water to 50 mL. The glass flask containing direct enzymatic hydrolyzed juice was equipped with a purge head. One milliliter of internal standard (50 µL/L 4-methyl-1-pentanol) was added. Sample temperature was equilibrated in a 30°C water bath for 10 min. Under magnetic stirring (200 rpm) the sample was then purged with nitrogen (100 mL/min) for 60 min. The volatiles were collected into traps containing 250 mg of Tenax TA (mesh size = 60/80, Buchem bv, Apeldoorn, The Netherlands). **Quantification.** Quantification of the direct enzymatic hydrolysis samples was carried out on reference aroma compounds (200mg/L) in the model solution. For dynamic headspace collection 50 mL of model solution and 0.1M citrate-phosphate buffer (pH 5) (1:1) adjusted to pH 5 was used.

Gas Chromatography-Mass Spectrometry (GC-MS). From the enzymatic experiments the collected volatiles were thermally desorbed using an Automated Thermal Desorber (ATD 400, Perkin Elmer, USA) and separation and identification of aroma compounds was carried out on a Hewlett-Packard (Palo Alto, CA) G1800A S GC-MS system equipped with a J & W Scientific DB-Wax column (30 m x 0.25 mm x 0.25 µm). Settings were the same as described by (*8*). From the heating experiments two µL of the extracted volatiles were injected and separation and identification of aroma compounds were carried out on a Hewlett-Packard (Palo Alto, CA) G1530A S GC-MS System with the same parameters as described above. Identifications were carried out by probability-based matching with mass spectra in the G1035A Wiley library (Hewlett-Packard,Palo Alto, CA), and comparisons with mass spectra and retention indices (RI) of authentic reference standards. Linear retention indices were calculated after analysis under the same conditions of an *n*-alkane series (C9-C24). Peak area calculations were based on single ions, and peak areas of aroma compounds were divided by peak area of the internal standard.

RESULTS AND DISCUSSION

Analysis of Terpenes in Black Currant Juice. Terpenes in black currant juice were determined by GC-MS after isolation by either solvent extraction or dynamic headspace collection (**Table 1**). Other compounds such as alcohols, esters, phenols and furans were also identified in the black currant juice (not shown). More terpene compounds were recovered by dynamic headspace collection than by solvent extraction as previously reported (*8*) (**Table 1**). However in this study, solvent extraction was used for isolation of aroma in the thermally treated samples since some of the compounds of interest, in particular terpene hydrocarbons, have been shown to decompose and rearrange on the adsorbent material during headspace sampling (*17*).

Reactivity of Terpenes during Thermal Treatment of Black Currant Juice and Model Solution. The stability and reactivity of selected terpenes (limonene, α-terpinene, linalool, α-terpineol, 4-terpineol and menthol) were studied by adding the pure compounds to either black currant juice or a model system and heating the samples for 30 min at 90 °C. The model system was designed to have the same acidity (pH = 3.1), °Brix-value and concentration of citric acid, glucose and fructose as black currant juice. Thermal treatment of black currant juice has been shown to cause changes in the concentration of all the selected terpenes except menthol (*7*). Linalool, α-terpineol and 4-terpineol have been shown to be of importance to the odor of black currant juice by GC-O (*1,8,9*).

The thermal treatment led to decreased concentrations of the terpenes added to the model solutions (**Table 2**). Menthol was the most stable of the studied terpenes with a loss of only 5 %. 4-Terpineol and α-terpineol were degraded by 29 and 21 %, respectively. Isocineole was formed from the latter two compounds, and γ-terpinene was also formed from 4-terpineol.

Limonene and linalool were degraded to similar extends, 69 and 62 % respectively. α-terpineol was formed from both, and nerol and geraniol was formed in addition from linalool. α-Terpinene was subject to the most extensive degradation (92 %), but no conversion products were detected. In the case of linalool most of the loss could be accounted for, but generally the loss of compounds exceeded the detected increases in concentrations of products formed.

The thermal treatment of pure terpenes added to black currant juice resulted in losses similar to what were observed with the model system. The same and small amounts of some additional products were formed, α-terpineol being a main conversion product of most of the compounds (**Table 2**). The loss of 4-terpineol, and most of the linalool loss could be accounted for by increased concentrations of other tepernes. However, even though more of the loss could generally be accounted for in the juice system than in the model system, an over all loss of compounds occurred. An exception from this was menthol, where somewhat more products were formed than lost.

The heat induced losses of added terpenes in the model solution and in black currant juice were closely related (**Figure 1**). Also similar patterns of products formed were observed in the two systems. The ability of the model system to simulate the behavior of the terpenes in black currant juice during heating, suggest that no other major components of the juice than the model system parameters selected, i.e. sugars, acid content, pH and °Brix, influences the degradation of the terpenes.

The main conversion product of linalool and limonene in stored model solutions of orange juice was also α-terpineol, and in addition cis-1,8-*p*-menthanediol was formed. Linalool also rearranged to nerol and geraniol (*18*). Under acidic conditions nerol and geraniol in terms cyclize to form α-terpineol and terpene diols (*19*). In a model citrus juice solution was α-terpineol converted to unidentified products faster than it was formed from both linalool and limonene and its formation was strongly pH and temperature dependent. The formation of α-terpineol from linalool was faster than its formation from limonene (*20*). The primary product of the acid-catalyzed hydration of limonene was also in other studies reported to be α-terpineol, but at elevated temperatures β-terpineol and terpinolene (*21*) as well as 1,8-*p*-menthanediol (*10*) was formed in addition. 1,8-*p*-menthanediol and other terpene diols are reported to form from α-terpineol and 4-terpineol by acid catalyzed hydration and further transformation to cineole or isocineole (*10,20,22,23*). According to a review by Clark and Chamblee (*10*) little work has been done on the reactions of α-terpinene**,** since it is relatively stable due to slower rates of hydration for conjugated double bonds in aqueous acids. In a study of thermal treatment of α-terpinene with aqueous acid (*21*) no evidence of hydration was observed, but it in part oxidized to *p*-cymene.

In the present study a possible formation of terpene diol intermediates similar to the reported 1,8-*p*-menthanediol might explain the overall loss of products observed during heating of some of the terpenes. Terpene diols might not be extracted into the ether/pentane phase as suggested by (*10*), and due to their low volatility these components might not be recovered by headspace collection either. The use of more polar solvents might lead to improved recovery of this type of products (*24*).

Glycosidically Bound Terpenes in Black Currant Juice. Heat treatment could potentially lead to a release of terpenes bound as glycosides thus increasing the amount of volatile terpenes. The amount and identity of glycosidically bound terpenes in black currant juice was examined by the enzymatic release of terpenes from glycoside isolates obtained by

Amberlite XAD-2 fractionation of black currant juice and from direct enzymatic treatment of the juice.

Several terpenes in black currant juice were found to be bound to glycosides. Seven terpene alcohols and aldehydes were identified after enzymatic treatment of the chromatographically prepared extract with β-glucosidase (**Table 3**). No volatile compounds were released from a control sample of the glycosidic extract. Hence, the other terpenes identified in black currant juice (see **Table 1**) do not form glycosides. Direct enzymatic treatment of black currant juice with β-glucosidase gave the same pattern of released volatile aglyconic compounds (**Table 3**). However, the concentration levels are not directly comparable due to the different methods used. *p*-Menthene-9-al and 3-caren-10-al were identified in the glycosidic fraction only. The glycosidically bound fraction was larger than the free fraction for all compounds except linalool oxide. Cumin aldehyde was the compound found in the highest concentration as glycosidically bound. Also some glycosidically bound alcohols were found in the black currant juice as described elsewhere (*15*). Some of the identified glycosidically bound terpenes have been reported as aglycones in other fruits (*25*).

Relationship to Terpene Changes Observed during Heating and Concentration Studies. Several terpenes have previously been found to increase in concentration in black currant juice during heat application (*5-7,11,13*), but only linalool oxide and cumin aldehyde were found to be glycosidically bound in the present study. Evidence of aromatic glycosides in black currant berry has previously been indicated by Marriott (*14*) who found monoterpene alcohols and their corresponding alkenes in a ratio of 5:1. In that study the predominant aglyconic terpene alcohols were 4-terpineol and α-terpineol, as well as evidence of *p*-cymene-8-ol was found. In the present study none of these compounds were found to be glycosidically bound. The terpene alcohols linalool, 4-terpineol and α-terpineol identified in black currant juice are glycosidically bound in many other fruits (*25*), hence these and other bound compounds might have been released during earlier steps of the juice processing. However, none of the identified glycosidically bound terpenes have been reported to be of importance to the odor of black currant juice (*1,8,9*), and the release of free terpenes from a pool of glycosides are therefore not expected to be of major importance for the heat induced changes of the aroma of black currant juice.

The increase of linalool oxide observed by thermal treatment of black currant juice (*5,7,11*) can in addition to a possible glycosidic release originate from heat induced conversion of 4-terpineol or α-terpinene. Likewise, the increase in concentration of α-terpineol observed by (*7,11*) could be caused by acid catalyzed conversion of various terpenes. On the other hand, the decrease of linalool (*7,13*) may be explained by the rearrangement and cyclisation into other terpineols. The increase of *p*-cymene, γ-terpinene, α-terpinene and α-terpinolene observed in (*7,11,13*) arise from degradation of other terpenes.

In conclusion, the terpene changes observed during thermal treatment of an aqueous acidic system like black currant juice can be explained mainly by acid catalyzed terpene reactions and to a minor degree glycosidic release. The reactions are part of a complex system where compounds are formed and degraded simultaneously.

ABBREVIATIONS USED

GC-O, gas chromatography-olfactometry; GC-MS, gas chromatography-mass spectrometry; RI, retention index; min, minutes.

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TABLES

Table 1. Terpenes identified in black currant juice.

compound[b]	RI	isolation method, occurrence[a]	
		dynamic headspace	solvent extraction
α-pinene[c]	1008	-	+
α-terpinene[c]	1167	+	+
isocineole[c]	1169	+	+
limonene[c]	1184	+	+
cineole[c]	1194	+	+
γ-terpinene[c]	1223	+	+
p-cymene[c]	1253	+	+
α-terpinolene[c]	1266	+	+
rose oxide[c]	1337	+	-
menthone[c]	1438	+	-
linalool oxide[c]	1451	+	+
isomenthone[d]	1465	+	-
linalool[c]	1524	+	+
bornyl acetate[c]	1554	+	-
neomenthol [d]	1574	+	-
4-terpineol[c]	1577	+	+
p-menthene-9-al[d]	1584	+[e]	-
β-cyclocitral[c]	1590	+	-
menthol[c]	1618	+	+
1,8-menthadien-4-ol[d]	1662	+	+
α-terpineol[c]	1674	+	+
phellandral[d]	1696	+	-
3-caren-10-al[d]	1711	+[e]	-
cumin aldehyde[c]	1716	+	-
citronellol[c]	1750	+	-
β-damascenone[c]	1800	+	-
p-cymen-8-ol[d]	1824	+	-
trans-geraniol[c]	1830	+	-
exo-2-hydroxycineol[d]	1845	-	+

[a] (+) compounds identified; (-) not detected. [b] Other compounds identified in black currant juice see e.g. (*7*). [c] Mass spectra and RI agreed with authentic standards. [d] Tentatively identified, mass spectrum agreed with the Wiley library. [e] Only identified in the glycosidic fraction.

Table 2. Terpene degradation during thermal treatment (90 °C for 30 min).

	model solution		black currant juice	
terpene standard	loss (%)	conversion products[a]	loss (%)	conversion products[a]
α-terpinene	92		86	α-terpineol (5%)[b], linalool oxide (4%), 4-terpineol (4%), isocineole (1%), α-terpinolene (1%)
limonene	69	α-terpineol (1%)[c]	75	α-terpineol (3%), *p*-cymene (1%)
linalool	62	α-terpineol (31%), geraniol (18%), nerol (6%)	78	α-terpineol (39%), geraniol (12 %), nerol (4%)
4-terpineol	29	isocineole (3%), γ-terpinene (1%)	28	α-terpinene (8%), α-terpineol (6%), isocineole (6%), linalool oxide (4%), γ-terpinene (3%), *p*-cymene (1%), α-terpinolene (1%)
α-terpineol	21	isocineole (1%)	21	α-terpinolene (2%), isocineole (1%)
menthol	5		0.4	α-terpineol (3%), α-terpinene (2%) linalool oxide (2%), *p*-cymene (2%), α-terpinolene (1%), isocineole (1%)

[a]Compounds constituting ≥1% of the initial terpene standard concentration (n=3); CV (%)<20. [b](Concentration of the conversion product in the heated juice added terpene - concentration of conversion product in heated juice)/concentration of the terpene in the non heated model system*100. [c]Concentration of the conversion product in the heated model system/concentration of the terpene standard in the non heated model system*100.

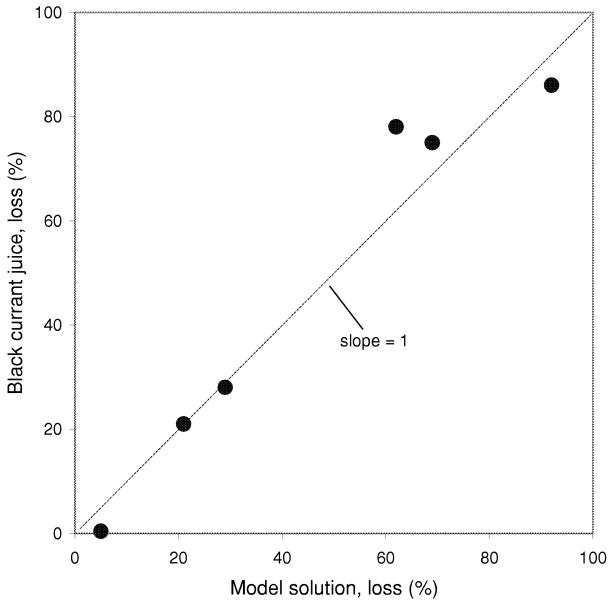
Table 3. Glycosidically bound monoterpenes in black currant juice.

		direct enzymatic hydrolysis of black currant juice		
compound	enzymatic hydrolysis of glycosidic extract (µg/L)[b]	free[a] (µg/L)[c]	bound (µg/L) [d]	bound (%)
linalool oxide	25	24±2	5	19
neomenthol[e]	11	1±0	14	95
p-menthene-9-al[f]	23	0±0	9	100
menthol	5	7±0	11	60
3-caren-10-al[g]	3	0±0	3	100
cumin aldehyde	441	80±8	313	80
geraniol	30	11±2	52	83

[a] Refer to **Table 1** for other free terpene compounds identified in the juice. [b] Values are not directly comparable with those of direct enzymatic hydrolysis (n=1). [c] Concentrations are given as average ± standard deviation (n=3). [d] Concentration of enzymatically hydrolyzed juice – concentration of untreated juice. [e] Quantified on the basis of menthol standard. . [f] Quantified on the basis of β-cyclocitral standard. [g] Quantified on the basis of camphene standard.

FIGURES

Figure 1. Relationship between thermally induced (90 °C/30 min) losses of terpenes in model solution and when added to black currant juice.



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Paper VIII

Glycosidically bound alcohols of blackcurrant juice

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Varming, C., Andersen, M. L. and Poll, L.

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Camilla Varming, Mogens L. Andersen, Leif Poll

Department of Food Science, The Royal Veterinary and Agricultural University, Rolighedsvej 30, 1958 Frederiksberg C, Denmark

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1. ABSTRACT

The release of bound volatile alcohols of blackcurrant juice from the corresponding glycosides was studied enzymatically using β-glycosidase in two different ways. β-glycosidase was added either directly to the juice, or to an extract of glycosidic compounds that had been isolated from the juice by chromatography using Amberlite XAD-2. The two methods resulted in the same patterns of released volatile aglyconic compounds. Fifteen aliphatic alcohols, four aromatic alcohols and one aromatic aldehyde were identified in the glycosidically bound fraction.

2. INTRODUCTION

Many fruits contain non-volatile precursors of aroma compounds in the form of glycosidically bound compounds [1]. Volatile aroma compounds can be released from the precursors by the action of e.g. heat, acid hydrolysis or enzymes. This has been a subject of interest especially in grapes and wine, whereas the presence of glycosidically bound aroma components in blackcurrant has previously only been indicated [2]. Heating and other processing of blackcurrant berries and juice cause changes in the levels of aroma components [3-6]. The possibility that some of the observed changes are caused by release from a pool of glycosidically bound aroma compounds is investigated in the present study.

3. MATERIALS AND METHODS

Materials

Commercial blackcurrant *(Ribes nigrum* L.) juice of the variety Ben Lemond was obtained from an industrial plant as described by [4] and kept at –18°C until analysis. 0.1 M citrate-phosphate buffer (pH 5) and almond ß-glucosidase enzyme solution (10 mg/ml, Sigma-Aldrich, Denmark) in buffer were used.

Enzymatic hydrolysis

Enzymatic hydrolysis of chromatographically separated glycosidic extract: Free and glycosidically bound aroma compounds from 100 ml juice were chromatographically separated on an XAD-2 column, according to the method described in [7]. Water soluble components were eluted with 50 mL of distilled water, and the free volatile fraction was extracted with 50 ml pentane/diethylether (1:1) and discharged. The glycosidically bound aroma fraction was

extracted with 50 ml methanol, concentrated under reduced pressure to approx. 1 ml and redissolved in 2.0 ml buffer. Half of this glycosidic extract was added 2.0 ml β-glucosidase solution in a small vial. The other half of the glycosidic extract was added 2.0 ml buffer, constituting the control sample.

Direct enzymatic hydrolysis: Blackcurrant juice and buffer (1:1) was adjusted to pH 5. Fifty milliliter of the mixture was transferred to a 250 ml glass flask equipped with a lid and 2.0 ml of the ß-glucosidase solution was added. The juice sample not to be enzymatically treated constituted 50 ml of the juice and buffer (1:1) and 2.0 ml buffer.

All samples were incubated for 19h in a 37°C water bath.

Determination of volatile compounds

Dynamic headspace sampling was carried out immediately after the enzyme treatments. The chromatographically separated glycosidic extract sample was transferred to a glass flask equipped with a purge head and diluted with water to 50 ml. The glass flask containing direct enzymatically hydrolysed juice was equipped with a purge head. One milliliter of internal standard (50 µl/l 4-methyl-1-pentanol) was added. Sample temperature was equilibrated in a 30°C water bath for 10 min. Under magnetic stirring (200 rpm) the sample was then purged with nitrogen (100 ml/min) for 60 minutes. The volatiles were collected on Tenax TA traps.

Quantification of the direct enzymatic hydrolysis samples was carried out on reference aroma compounds (200 ppb) in a model solution (glucose conc. 48 g/l, fructose conc. 61 g/l, citric acid monohydrate conc. 39 g/l in water, 14°B) resembling blackcurrant juice. For dynamic headspace sampling 50 ml of model solution and buffer (1:1) adjusted to pH 5 was used.

An Automated Thermal Desorber and a GC-MS system were used as described by [8]. Identification was carried out by probability-based matching with mass spectra in the G1035A Wiley library (Hewlett-Packard). Peak area calculations were based on single ions, and peak areas of aroma compounds were divided by peak area of the internal standard.

4. RESULTS AND DISCUSSION

Glycosidically bound compounds in blackcurrant juice were isolated using an Amberlite XAD-2 column. Fifteen aliphatic alcohols, four aromatic alcohols as well as an aromatic aldehyde were identified upon enzymatic treatment of the chromatographically prepared extract with β-glycosidase (Table 1). No volatile compounds were released from a control sample of the glycosidic extract. Also some glycosidically bound terpenoids were found which will be described in a separate paper [9].

Direct enzymatic treatment of blackcurrant juice with β-glucosidase gave the same pattern of released volatile aglyconic compounds, except for 3-methyl-1-butanol (Table 1). However, the concentration levels are not directly comparable due to the different methods used.

Table 1. Free and glycosidically bound volatile alcohols in blackcurrant juice.

Compound[a]	Enzymatic hydrolysis of glycosidic extract (µg/l)[b]	Direct enzymatic hydrolysis of blackcurrant juice			
		Free (µg/l)[c]	Bound[d] (µg/l)	% Free	% Bound
2-butanol	59	6±2	33	15	85
2-methyl-1-propanol[e]	64	431±13	0	100	0
3-pentanol	42	4±0	55	6	94
2-pentanol	219	22±1	226	9	91
1-butanol	100	954±47	67	93	7
1-penten-3-ol	70	26±10	107	20	80
3-methyl-1-butanol	1042	3535±100	17	100	0
3-methyl-3-buten-1-ol	43	7±1	30	19	81
1-pentanol	30	18±1	35	35	65
1-hexanol	242	97±4	206	32	68
Cis-3-hexen-1-ol	117	29±4	81	26	74
trans-2-hexenol	16	4±1	10	29	71
1-octen-3-ol	15	12±1	6	65	35
1-heptanol	3	3±1	3	50	50
1-octanol	14	9±0	12	42	58
methyl salicylate	199	11±1	137	7	93
benzyl alcohol	1313	60±65	1934	3	97
eugenol	197	284±124	280	50	50
2,4-bis-tert-butylphenol[f]	2	2±0	1	57	43
benzaldehyde	209	117±13	111	51	49

[a] Other compounds identified in blackcurrant juice see eg [4]. [b](n=1) Values are not directly comparable with those of direct enzymatic hydrolysis. [c] Concentrations are given as average ± standard deviation (n=3). [d] Concentration of enzymatically hydrolysed juice – concentration of untreated juice. [e] Break through on the adsorbent traps. [f] Quantified on the basis of thymol standard.

A large variation in the amount and proportion of the glycosidically bound volatiles in the blackcurrant juice was seen, and no obvious pattern is recognized. For most compounds the glycosidically bound fraction was larger than the free fraction, but all compounds identified as glycosidically bound were also present in the free form. Benzyl alcohol was the glycosidically bound compound that had the highest ratio between bound and free amount (bound concentration was 32 times the free concentration) followed by eugenol, 2-pentanol, and 1-hexanol. Existence of aromatic glycosides in blackcurrants has previously been indicated [2] and most of the glycosidically released compounds have been found as aglycones in other fruits also [1].

Results of the present study confirm that the increase of aromatic alcohols observed by thermal treatment [4] is likely to be, at least partly, caused by a heat induced glycosidic release. Also during the concentration of blackcurrant juice an increase of benzaldehyde, benzyl alcohol, eugenol, and methyl salicylate is found [5,6]. In neither of these studies did the concentration of aliphatic alcohols however increase, indicating that the applied heat does not

induce a glycosidic release of these compounds. Possibly, glycosidically bound volatiles could have been released during other steps of the juice processing [3].

Some of the identified glycosidically bound alcohols are important of the odour of blackcurrant juice, i.e. 3-methyl-1-butanol, cis-3-hexen-1-ol, 1-octen-3-ol, 1-octanol, benzyl alcohol, and eugenol [3,6,8]. Glycosidic release might result in additional compounds having concentrations exceeding their odor threshold values. Hence, release from a pool of glycosidically bound alcohols by enzymatic hydrolysis can lead to changes in the sensory impression of blackcurrant juice.

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