Nora Pap

VALUE-ADDED PROCESSING OF BLACKCURRANTS

USE OF MEMBRANE TECHNOLOGIES FOR CLARIFICATION AND CONCENTRATION OF BLACKCURRANT JUICE AND EXTRACTION OF ANTHOCYANINS FROM BLACKCURRANT MARC
NORA PAP

VALUE-ADDED PROCESSING OF BLACKCURRANTS
Use of membrane technologies for clarification and concentration of blackcurrant juice and extraction of anthocyanins from blackcurrant marc

Academic dissertation to be presented with the assent of the Doctoral Training Committee of Technology and Natural Sciences of the University of Oulu for public defence in the OP-Pohjola auditorium (L6), Linnanmaa, on 16 November 2018, at 12 noon

UNIVERSITY OF OULU, OULU 2018
Abstract

Blackcurrants (Ribes nigrum L.) are widely consumed due to their favourable taste and health-promoting effects. The berries and extracts from different parts of the plant show anticarcinogenic, antioxidative and anti-inflammatory properties, and are effective in reducing the risk of cardiovascular disease and in promoting brain health. These health-promoting benefits are due to high concentrations of valuable compounds such as anthocyanins and flavonols in blackcurrants. However, these compounds are sensitive to heat and processing and some are lost when the berries are processed into products such as jams, purees and juices.

Industrial processing of juices is a multistep process that typically includes enzyme treatment, pressing, pasteurisation, clarification and usually also thermal concentration. Alternative minimal processing technologies are required to preserve the health-promoting compounds in products by avoiding the use of high temperatures and extensive clarification.

Integrated membrane technology, i.e. combined ultrafiltration and reverse osmosis, was used in this thesis for the production of blackcurrant juice concentrate. Pre-treatment methods, such as enzymatic treatment, ultrafiltration, enzymatic treatment combined with ultrafiltration and centrifugation to increase the filtration efficiency in reverse osmosis were evaluated. Processing was modelled to define the resistances, using the resistance-in-series model. The preservation and concentration of anthocyanins and flavonols were analysed. The results indicated that the main resistance in the reverse osmosis process was polarisation resistance, while membrane resistance was lower and fouling resistance was one order of magnitude lower than the other resistances.

The filtration efficiency results showed that the highest flux was achieved by ultrafiltered blackcurrant juice, but that the resulting juices were substantially lower in anthocyanins and flavonols, which were retained on the ultrafiltration membrane. Therefore, replacing ultrafiltration with centrifugation as the clarification method for juices is recommended.

Value-added processing of blackcurrant was conceptualised by valorisation of the marc left in the berry pressing process for extraction of anthocyanin compounds. Conventional extraction was compared with microwave-assisted extraction (MAE), with the latter optimised using response surface methodology to achieve maximum efficiency in extracting anthocyanins. The optimum parameters found for MAE were: microwave power 700 W, extraction time 10 minutes, pH 2 adjusted with hydrochloric acid and a solid to solvent ratio of 0.05. Conventional extraction showed the best results when carried out at 80 °C for 300 minutes in aqueous solution with pH 2 adjusted by hydrochloric acid. Under these conditions, recovery of anthocyanins was still 10% lower than with MAE for only 10 minutes of extraction time.

Keywords: anthocyanins, blackcurrant, conventional extraction, flavonols, microwave-assisted extraction, reverse osmosis, ultrafiltration, value-added processing
Pap, Nora, Lisäärvon tuotto mustaherukan prosessoinnissa. Mustaherukkamehun konsentointi ja kirkastus kalvosuodatustekniikoilla sekä antosyaanien uutto mustaherukan puristekakusta

Oulun yliopiston tutkijakoulu; Oulun yliopisto, Teknillinen tiedekunta

*Acta Univ. Oul. C* 680, 2018
Oulun yliopisto, PL 8000, 90014 Oulun yliopisto

**Tiivistelmä**

Mustaherukoita käytetään paljon niiden hyvän maun ja terveyttä edistävien ansios- ta. Marjoilla ja marj akasvin eri osien uutilla on osoitettu olevan antikarsinogeneenisia, antioksi- datiivisia ja tulehduksia estäviä ominaisuuksia ja ne ovat tehokkaita pienentämään sydän- ja verisuonisairauksia. Ne edistävät myös aivojen terveyttä. Marjojen arvokkailla yhdisteillä kuten antosyanideillä ja flavonoleilla on terveysyödyllisiä yhdisteitä, koska ne ovat herkkiä lämmölle ja prosessoinnin vaikutuksille.

Mehujen prosessoinnissa käytetään entsyymikäsittelyjä, puristusta, pastörointia, selkeytystä ja usein myös lämpökonsentrointia. Tuotteiden terveyttä edistävien yhdisteiden säilyttämiseksi tarvitaan uudenlaisia hellävaraisia prosessointitekniikoita ilman korkeita lämpötiloja ja voimakasta selkeyttämistä.


Mustaherukkamuhun tuotannossa muodostuu sivutuotteen a ns. puristekakkua, joka sisältää runsaasti antosyananeja. Työssä kehitettiin antosyaanien talteenottoa tästä sivutuotteesta vertaa- mallia tavanomaisesta uutotecistuksiikaa mikroaaltoavousteiseen uuttoon. Prosessi optimoitiin vastepintamenetelmällä mahdollisimman suuren antosyaanien uutotekohkkujen saavuttamiseksi.

Optimaaliset parametrit saatiin mikroaaltoavousteksisessa uotossa teholla 700 W, uuttoajalla 10 minuuttia, kiintoaines-liuotin -suhteella 0,05 pH-arvossa 2, mikä saavutettiin lisäämällä suola- happoa. Tavanomaisessa uotossa parhaat antosyaanisaannot saavutettiin 0,5 pH-arvossa 2, mikä saavutettiin suositellun suolahappo-vesiliuoksella pH-arvossa 2 uuttamalla 30 minuuttia lämpötilassa 80 °C. Antosyaanisaanto oli kuitenkin tavanomaisessa uotossa optimiolosuhteissa 10% pienempi kuin mikroaaltoavousteksisessa uotossa 10 minuutin uuttoajalla.

**Asiakas:** antosyanidit, flavonolit, käänteisosmoosi, lisäärvon tuotto mustaherukkaprosessissa, mikroaaltoavoustekineinen uutto, tavanomainen uutto, ultrasuodatus

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**Tiivistelmä:**

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To my beloved kids, Jessica and Joel
Acknowledgements

This doctoral research was conducted at the University of Oulu, Environmental and Chemical Research Unit, in Finland.

First of all, my greatest gratitude to my supervisors, to Professor Riitta Keiski and to Professor Eva Pongrácz, for giving me the possibility to work under their guidance. Your continuous support, encouragement and advices were invaluable and I am deeply thankful for it.

Special thanks to my advisor, Lic.Sc. (Tech.) Liisa Myllykoski, for her valuable help during my doctoral studies and for guidance in the research in the field of food industry.

I would like to thank Docent Mari Kallioinen from Lappeenranta University of Technology in Finland and Professor Željko Knez from the University of Maribor in Slovenia for reviewing my thesis, and for improving the quality with their valuable comments.

During my research, I had the opportunity to work both in national and international research groups.

Professor Vesa Virtanen, Ph.D. Mari Jaakkola and M.Sc. Mika Mahosenaho at the Sotkamo Biotechnology Laboratory are appreciated for their co-operation.

I express my acknowledgement to Professor Cecília Hodúr at the University of Szeged and to Professor Gyula Vatai at the Szent István University in Hungary for introducing the world of membrane science and microwave-assisted extraction to me, and for the fruitful collaboration and discussions regarding the work in this thesis.

My colleagues Dr. Sándor Beszédes and Dr. Szabolcs Kertész at the University of Szeged are thanked for their invaluable advice and for being my friends for so many years now.

Assistance provided by Ms. Auli Turkki at the University of Oulu is greatly appreciated. All the advice given by You has been a great help in finalizing this thesis.

I am most grateful to Ph.D. Sari Mäkinen, Ph.D. Anne Pihlanto and Ph.D. Pirjo Mattila for their endless trust in me and helping me whenever it was possible. I also thank M.Sc. Jarkko Hellström for being such a great office-mate and for all the professional support.

My team leader in the Industrial Symbiosis team, M.Sc. Juha-Matti Katajajuuri, and my group leader in the Biorefinery and Bioproducts group, PhD. Riitta Laitinen are greatly acknowledged for their support and understanding. The
vice president of the Production Solutions unit, D.Sc. (Tech.) Leena Paavilanen, is greatly acknowledged.

I wish to express my gratitude to Professor Antti Asikainen, Ph.D. Eila Järvenpää and D.Sc. (Tech.), Docent Mika Huhtanen for their support and their input as the members of the follow-up group of the thesis.

The financial support of the Graduate School in Chemical Engineering, Olvi Foundation, Finnish Food Research Foundation, Finnish Cultural Foundation, University of Oulu Foundation, Tauno Tönning Foundation, Thule Institute, Raisio Research Foundation and the National Resources Institute Finland is greatly acknowledged.

My sincere gratitude to my true friend, Ms. Imola Kovács, for her advices and guidelines even in those heavy storms of life, and for comforting me whenever I had a chance to stop over in Budapest.

I wish to thank to all my friends in Estonia, Finland, Hungary, Spain and Sweden for their support, for being available all the time for me, and for the great talks we had to forget about all troubles for a second.

The Aikoma family is greatly acknowledged. I am grateful for Saija, Janne, Esa and Tomi Aikoma, and for Maarit Laamanen for helping me to find the time for this thesis work.

My heart is full of gratitude when I think of my parents, Rózsa and Árpád. Your support and love are extremely important for me. You helped me through all obstacles to make my dream true of coming to Finland to conduct this research work!

My compliments to my sister, Timea and her family for their love and encouragement.

I also thank my Kummi-family Mielikäinen in Kuusamo for all the support they provided me from the beginning of my professional career.

A heartfelt thanks for my children, Jessica and Joel for their amazing patience, encouragement and endless faith in me! You are my sunshine, I love you so much!

October 2018

Nora Pap
Symbols

\( C_{P, \, \text{Perm}} \)  \quad \text{concentration of the compound in the permeate (g/L or kmol/m}^3\))

\( c_M \)  \quad \text{concentration of the compound at the membrane surface (kg/m}^3\))

\( c_R \)  \quad \text{concentration of the compound in the retentate (g/L or kmol/m}^3\))

\( J_F \)  \quad \text{water flux measured at fixed temperature (L/m}^2\cdot\text{h})

\( J_W \)  \quad \text{pure water flux (L/m}^2\cdot\text{h})

MLR  \quad \text{multiple linear regression}

\( P_{\text{in}} \)  \quad \text{inlet pressure of the membrane module (bar)}

\( P_{\text{out}} \)  \quad \text{outlet pressure of the membrane module (bar)}

\( P_{\text{perm}} \)  \quad \text{pressure on the permeate side (bar)}

\( Q \)  \quad \text{recirculation flow rate (L/h)}

\( Q^2 \)  \quad \text{fraction of the variation of the response}

\( R^2 \)  \quad \text{fraction of the variation}

\( R \)  \quad \text{retention of the membrane in Eq. 4 (\%)}

\( R \)  \quad \text{universal gas constant in Eq. 9 (J/kmol/K)}

\( R_C \)  \quad \text{cake layer resistance (/m)}

\( R_F \)  \quad \text{fouling resistance (/m)}

\( R_M \)  \quad \text{membrane resistance (/m)}

\( R_P \)  \quad \text{polarisation layer resistance (/m)}

\( R_T \)  \quad \text{total resistance (/m)}

\( T \)  \quad \text{temperature (K)}

\( \tan \delta \)  \quad \text{dissipation factor}

\( V_0 \)  \quad \text{volume of the feed (L)}

\( V_P \)  \quad \text{volume of the permeate (L)}

\( V_R \)  \quad \text{volume of the retentate (L)}

\( V_{RF} \)  \quad \text{volumetric reduction factor}

\( x_i \)  \quad \text{independent variables of the model}

\( Y \)  \quad \text{dependent variable}

\( \beta \)  \quad \text{concentration polarisation}

\( \beta_j \)  \quad \text{regression coefficient of the model}

\( \Delta P \)  \quad \text{transmembrane pressure (bar)}

\( \Delta P_{TM} \)  \quad \text{transmembrane pressure (bar)}

\( \Delta \pi \)  \quad \text{osmotic pressure (bar)}
\( \varepsilon \) noise observed in the response in Eq. 12
\( \varepsilon \) molar absorptivity in Eq. 14
\( \varepsilon' \) dielectric constant
\( \varepsilon'' \) dielectric loss
\( \eta \) viscosity of the permeate (Pas)
\( \eta_w \) water viscosity at 20 °C (Pas)
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>ASE</td>
<td>accelerated solvent extraction</td>
</tr>
<tr>
<td>CCF</td>
<td>central composite face-centred design</td>
</tr>
<tr>
<td>CEx</td>
<td>conventional extraction</td>
</tr>
<tr>
<td>CE</td>
<td>centrifugation in page 75</td>
</tr>
<tr>
<td>Cya</td>
<td>cyanidin</td>
</tr>
<tr>
<td>cya-3-glu</td>
<td>cyanidin-3-glucoside</td>
</tr>
<tr>
<td>cya-3-rut</td>
<td>cyanidin-3-rutinoside</td>
</tr>
<tr>
<td>Da</td>
<td>Dalton</td>
</tr>
<tr>
<td>DAD</td>
<td>diode array detector</td>
</tr>
<tr>
<td>DF</td>
<td>dilution factor</td>
</tr>
<tr>
<td>Dp</td>
<td>dephinidin</td>
</tr>
<tr>
<td>dp-3-glu</td>
<td>delphinidin-3-glucoside</td>
</tr>
<tr>
<td>dp-3-rut</td>
<td>delphinidin-3-rutinoside</td>
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<tr>
<td>EDTA</td>
<td>ethylenediamine tetra-acetic acid</td>
</tr>
<tr>
<td>glu</td>
<td>glucoside</td>
</tr>
<tr>
<td>GmbH</td>
<td>company with limited liability</td>
</tr>
<tr>
<td>HCl</td>
<td>hydrochloric acid</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>HTST</td>
<td>high temperature short time treatment</td>
</tr>
<tr>
<td>LTLT</td>
<td>low temperature, low time treatment</td>
</tr>
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<td>M</td>
<td>molar</td>
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<td>MAE</td>
<td>microwave-assisted extraction</td>
</tr>
<tr>
<td>mal</td>
<td>malonylg glucoside</td>
</tr>
<tr>
<td>mal-3-rut</td>
<td>malvidin-3-rutinoside</td>
</tr>
<tr>
<td>MAP</td>
<td>monomeric anthocyanin pigment</td>
</tr>
<tr>
<td>MD</td>
<td>membrane distillation</td>
</tr>
<tr>
<td>MDS</td>
<td>mass selective detector</td>
</tr>
<tr>
<td>MF</td>
<td>microfiltration</td>
</tr>
<tr>
<td>Mv</td>
<td>malvidin</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>MWP</td>
<td>microwave power</td>
</tr>
<tr>
<td>NaOH</td>
<td>sodium hydroxide</td>
</tr>
<tr>
<td>NaOCl</td>
<td>sodium hypochlorite</td>
</tr>
<tr>
<td>NF</td>
<td>nanofiltration</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
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<tr>
<td>NWCO</td>
<td>nominal weight cut-off value of the membrane (Da)</td>
</tr>
<tr>
<td>OD</td>
<td>osmotic distillation</td>
</tr>
<tr>
<td>OJ1</td>
<td>original blackcurrant juice 1st sample</td>
</tr>
<tr>
<td>OJ1+PA+CE</td>
<td>Panzym Super E-treated blackcurrant juice after centrifugation</td>
</tr>
<tr>
<td>OJ1+PA+CE+ROJ</td>
<td>Panzym Super E-treated, centrifuged, concentrated blackcurrant juice</td>
</tr>
<tr>
<td>OJ2</td>
<td>original blackcurrant juice 2nd sample</td>
</tr>
<tr>
<td>OJ2+RB+CE</td>
<td>Rohapect berry-treated blackcurrant juice after centrifugation</td>
</tr>
<tr>
<td>OJ2+RB+CE+ROJ</td>
<td>Rohapect berry-treated, centrifuged, concentrated blackcurrant juice</td>
</tr>
<tr>
<td>OJ3</td>
<td>original blackcurrant juice 3rd sample</td>
</tr>
<tr>
<td>OJ3+CE</td>
<td>centrifuged blackcurrant juice</td>
</tr>
<tr>
<td>OJ3+CE+ROJ</td>
<td>centrifuged, concentrated blackcurrant juice</td>
</tr>
<tr>
<td>PDA</td>
<td>photodiode array detector</td>
</tr>
<tr>
<td>PE</td>
<td>polyether</td>
</tr>
<tr>
<td>PECTU/mL</td>
<td>pectinesterase unit/mL</td>
</tr>
<tr>
<td>pel-3-glu</td>
<td>pelargonidin-3-glucoside</td>
</tr>
<tr>
<td>PES</td>
<td>polyethersulfone</td>
</tr>
<tr>
<td>Pg</td>
<td>pelargonidin</td>
</tr>
<tr>
<td>Pn</td>
<td>peonidin</td>
</tr>
<tr>
<td>PSE</td>
<td>Panzym Super E enzyme</td>
</tr>
<tr>
<td>Pt</td>
<td>petunidin</td>
</tr>
<tr>
<td>PTF/mg</td>
<td>pectin transeliminase activity</td>
</tr>
<tr>
<td>PTFE</td>
<td>polytetrafluoroethylene</td>
</tr>
<tr>
<td>PVPP</td>
<td>polyvinyl polypyrrolidone</td>
</tr>
<tr>
<td>RSM</td>
<td>response surface methodology</td>
</tr>
<tr>
<td>rut</td>
<td>rutinoside</td>
</tr>
<tr>
<td>S:L</td>
<td>marc to solvent ratio</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulphate</td>
</tr>
<tr>
<td>SFE</td>
<td>supercritical fluid extraction</td>
</tr>
<tr>
<td>SO₂</td>
<td>sulphur dioxide</td>
</tr>
<tr>
<td>TMP</td>
<td>transmembrane pressure</td>
</tr>
<tr>
<td>TSS</td>
<td>total soluble solids</td>
</tr>
<tr>
<td>UF</td>
<td>ultrafiltration</td>
</tr>
<tr>
<td>UFJ</td>
<td>blackcurrant juice after ultrafiltration</td>
</tr>
</tbody>
</table>
UFJ+ROJ  blackcurrant juice after ultrafiltration and reverse osmosis
USE  ultrasound-assisted extraction
v/v %  volume percent
w/w %  weight by weight percent
VRF  volumetric reduction factor
W  Watts
List of original articles

The thesis is based on the following original research papers:


These papers are the result of fruitful collaborations with national and international universities. Pap was the first author of all four papers. In Paper I, she designed and conducted the experiments, except the chemical analysis, which was performed in collaboration with the Biotechnology laboratory at the University of Oulu in Sotkamo, planned the content of the article and analysed the data, except for the statistical analysis, which was performed by the collaborating partners at the University of Szeged in Hungary, and wrote the article.

In Paper II, Pap planned and conducted the experiments, and planned the content of the paper. The modelling of the reverse osmosis was done in collaboration with the University of Szeged and Szent István University in Hungary.

In Paper III and Paper IV, Pap was responsible for the design of experiments, planning the article and for the writing process, while the analysis and statistical analysis were carried out at the Biotechnology laboratory at the University of Oulu in Sotkamo and the University of Szeged, Hungary, respectively.
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1 Introduction and scope of the study

Blackcurrant (Ribes nigrum L.) is the second most important cultivated berry in Finland, after strawberry. The most important varieties grown are Öjebyn and Mortti. It is estimated that the area on which blackcurrant is cultivated is about 2000 ha and the total amount produced in Finland is around 1.8 million kg. Around 0.2 million kg of domestic berries are used annually to produce juice concentrates. Apart from the juice concentrates, the juice industry also produces unavoidable by-products and wastes. Around 20-30% of the total berry mass is left behind in the pressing process as berry marc (Roininen & Mokkila, 2007), which contains skins, seeds and pulp.

These massive amounts of by-products produced annually are currently finding applications, mostly as feed and fertiliser, although the food industry also uses some of the by-products. In addition, wastewaters are produced in the manufacturing and cleaning process, the latter containing cleaning agents as contaminants. Depending on the product, the amount of wastewater can be 0.2-0.5 m³/product tonne (Peusa & Piilo, 2006).

Waste in the food industry includes e.g. wasted raw material, excessive use of water and chemicals for cleaning and used packaging materials. In addition, it includes other factors such as wasted time, effort and energy. Following the principles of the waste management hierarchy, the food industry can improve its environmental performance and also its cost-effectiveness. The waste management hierarchy (http://ec.europa.eu/environment/waste/framework/) is an order of preference of waste management options, where priority is given to waste prevention, which includes reduction at source, reduction of hazard and re-use. The next highest priority is preparing for re-use, followed by recycling and recovery. Disposal is the least preferred option. Waste prevention can be achieved through decreasing material losses and improving the efficiency of the process. This will result in economic savings due to avoided disposal costs, and will reduce the concentration and volume of the effluent that has to be discarded.

When reduction at source is not realised, the aim should be the recovery of valuable components. Valorisation can be defined as “the increase of technical and/or economic value of by-products and wastes that are generated in different agro-food industries” (Almorza et al., 2007). Valorisation techniques include different separation technologies, such as mechanical and mass transfer-based separation technologies, and sometimes chemical or biochemical modifications.
However, when a commercial company seeks to manage by-products and valorise them, it is not only an ecological question, but also an economic issue.

Today, more efforts are being devoted to achieving sustainability in different sectors of the bioeconomy. Moreover, consumer awareness is increasing and consumers are demanding products that are more environmentally friendly and have a well-preserved nutritional content. The Finnish food industry sector ranks the fourth among different industries, and recently it was estimated that the total increase in the food industry in revenue is 3% in 2018 when compared to the previous year (Tilastokeskus, 2018). The most important attribute of food production currently is the high percentage (82%) of the domestic content (Elintarviketeollisuusliitto, 2018), and in addition, the consumption of plant based food and especially plant based proteins are becoming more relevant.

The production and development of sustainable food products focus on environmental, economic and social issues. From the economic point of view, this thesis concentrates on the development of integrated processing technology concept with a maximum efficiency to produce value-added products from blackcurrants that are rich in anthocyanins and flavonols.

A concept, that is illustrated under the answer of research question 10 part at the end of the thesis, was developed for juice concentrate production and for extraction of anthocyanins from blackcurrant marc. From the environmental point of view, the focus is on waste minimisation and valorisation by implementing extraction techniques to recover valuable compounds from the by-product of the berry juice pressing. In the social dimension, the goal is to provide consumers with safe and health-promoting products, in the form of anthocyanin and flavonol rich juice concentrates, and anthocyanin extracts.

1.1 Scope of the thesis

The overall aim of this thesis was to provide novel information on the value-added processing of blackcurrants. The following research questions were set up:

1. How can the valuable components in blackcurrants be best preserved during processing?
2. What is the role of temperature in the initial pectinase treatment of blackcurrants?
3. What is the impact of enzyme dosage on the permeate flux in the blackcurrant juice clarification process?
4. Does the ultrafiltration alter the valuable compounds content of filtered blackcurrant juice?
5. Is ultrafiltration of blackcurrant juice prior to concentration by reverse osmosis necessary?
6. Which are the most important resistances that cause the drop in permeate flux when concentrating blackcurrant juice by reverse osmosis?
7. How does reverse osmosis perform in concentrating anthocyanins and flavonols in blackcurrant juice?
8. Does microwave-assisted extraction perform better than conventional extraction in extraction of anthocyanins from blackcurrant marc?
9. What are the optimum processing parameters in microwave-assisted extraction of anthocyanins from blackcurrant marc?
10. What is the best technology to achieve value-added processing of blackcurrants?

1.2 Contribution of the original publications

The thesis consists of four peer-reviewed articles (Papers I-IV). The contributions of these original publications to the aims of the work are illustrated in Figure 1.
Fig. 1. Contributions of the original publications.

Paper I: Ultrafiltration (UF) of blackcurrant juice
- What is the impact of the pectinase treatment and the operation temperature on permeate flux in UF?
- What is the impact of pectinase treatment and UF on anthocyanin and flavonol contents?

Paper II: Concentration of blackcurrant juice by reverse osmosis (RO)
- Which are the major membrane resistances affecting permeate flux?
- Which enzyme and process parameters should be applied in industrial applications?

Paper III: Concentration of blackcurrant juice by reverse osmosis (RO)
- Can clarification by UF be replaced with enzymatic treatment and centrifugation prior to RO without compromising efficiency?
- How are anthocyanins and flavonols affected in different process steps and are they concentrated in RO effectively?

Paper IV: Utilization of blackcurrant marc
- Is microwave-assisted extraction (MAE) more effective than conventional solvent (CE) extraction?
- What are the optimum processing parameters in MAE for maximum recovery of anthocyanins and is the composition altered when compared to CE?

Value-added processing of blackcurrants (Ribes nigrum L.)

Paper I focuses on the applicability of ultrafiltration as a clarification step in blackcurrant juice processing. The ultrafiltration stage was preceded by the depectinization of the juice by using pectinase preparation. The influence of the amount of enzyme used and the temperature applied during ultrafiltration on the permeate flux was evaluated. The effect of the ultrafiltration on the anthocyanin and flavonol contents was determined.

Paper II and Paper III deal with concentration of blackcurrant juice by reverse osmosis. In Paper II, the reverse osmosis process was modelled using the resistance-in-series model. Two commercial pectinase enzymes in different concentrations were used in depectinization of the blackcurrant juice to increase
the filtration efficiency, expressed as the permeate flux. The aim of the modelling of the ultrafiltration process was to aid the selection of an enzyme and processing conditions for industrial application.

The role of Paper III is to analyse whether the clarification by ultrafiltration can be substituted by enzymatic treatment and centrifugation without compromising the efficiency of the concentration process in terms of permeate flux. The effects of different processing steps on the anthocyanin and flavonol contents in the juice, as well as the efficiency of reverse osmosis to concentrate those in the final product are evaluated.

Paper IV investigates the feasibility of the by-product (blackcurrant marc) of the juice pressing process for extraction of anthocyanins in microwave-assisted and conventional extraction process. To have a maximum benefit of the microwaves, the extraction of anthocyanins from blackcurrant marc was optimized using response surface methodology and the efficiency of extraction was compared with that of a conventional solvent extraction process in terms of extraction time, anthocyanin concentration and possible change in composition in the extract.
2 Blackcurrant and its health-promoting flavonoid compounds

Blackcurrant is a medium-sized shrub growing to about 1.5-2.0 m high. It has been commonly grown in Europe since the 17th century (Doronina & Terekhina, 2017). It belongs to the family Grossulariaceae, genus *Ribes*. The Siberian subspecies (*R. nigrum* subsp. *sibiricum*) is usually distinguished from the European subspecies (*R. nigrum* subsp. *europaeum*). The mature berries are spherical, about 10 mm in diameter, usually dark black or dark-blue coloured. The colour is caused by the flavonoids present in the berries, especially anthocyanins.

Blackcurrants are consumed throughout the year in different forms because of their favourable nutritive value, which is described in Table 1 (Fineli, 2017).

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>310 kJ (74 kcal)</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>9.7 g</td>
</tr>
<tr>
<td>Protein</td>
<td>1.1 g</td>
</tr>
<tr>
<td>Fat</td>
<td>1.4 g</td>
</tr>
<tr>
<td>Vitamins</td>
<td></td>
</tr>
<tr>
<td>Folate</td>
<td>14.2 µg</td>
</tr>
<tr>
<td>Niacine</td>
<td>0.5 mg</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.02 mg</td>
</tr>
<tr>
<td>Thiamin (vitamin B1)</td>
<td>0.04 mg</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>128.0 mg</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>8.1 µg</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>542.2 µg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>2.2 mg</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>30.00 µg</td>
</tr>
<tr>
<td>Minerals</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>72.0 mg</td>
</tr>
<tr>
<td>Iron</td>
<td>1.2 mg</td>
</tr>
<tr>
<td>Iodide</td>
<td>1.0 µg</td>
</tr>
<tr>
<td>Potassium</td>
<td>340 mg</td>
</tr>
<tr>
<td>Magnesium</td>
<td>24.0 mg</td>
</tr>
<tr>
<td>Sodium</td>
<td>2.0 mg</td>
</tr>
<tr>
<td>Salts</td>
<td>5.1 mg</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>58.0 mg</td>
</tr>
<tr>
<td>Selenium</td>
<td>0.1 µg</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.3 mg</td>
</tr>
</tbody>
</table>
In addition to the polyphenol content, the quality of the fruit is highly dependent on the composition of the blackcurrant berry, especially the content of sugars, organic acids and vitamin C (Milivojević et al., 2009). The mineral content and the trace elements present in blackcurrants are essential for human health. Table 2 summarises the sugar content and the tartaric acid, malic acid and citric acid content of different cultivars of blackcurrant, as reported by Nour et al. (2011).

### Table 2. Quality-determining compounds in blackcurrants (based on Nour et al., 2011).

<table>
<thead>
<tr>
<th>Valuable compound</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugars</td>
<td>12.38 to 14.04%</td>
</tr>
<tr>
<td>Tartaric acid</td>
<td>31.31 to 112.94 mg/100 g fresh weight</td>
</tr>
<tr>
<td>Malic acid</td>
<td>95.10 to 303.94 mg/100 g fresh weight</td>
</tr>
<tr>
<td>Citric acid</td>
<td>2473.43 to 3553.31 mg/100 g fresh weight</td>
</tr>
</tbody>
</table>

The seeds of the berry are left behind in high amounts in the juice pressing process and, when separated from the skins and pulp, can be a valuable component for e.g. the cosmetics and dietary supplement industries. Flores & Ruiz del Castillo (2016) describe the composition of blackcurrant seeds as regards the total oil content, fatty acid composition and content of phenolic acids. The major fatty acid in the seeds is linoleic acid, which represents about 40-45% of total fatty acids. The α-linolenic acid, stearidonic acid and γ-linolenic acid concentration range is 12.9 -16.2%, 3.2- 4.5% and 16.2-18.8%, respectively, and is highly dependent on the cultivar.

The major phenolic acid in the seeds is p-coumaric acid, followed by gallic, ferulic and caffeic acid. These phenolic acids are of great importance in stabilisation of the fatty acids and, together with the fatty acids, they contribute strongly to the added-value of blackcurrant seeds.

### 2.1 Flavonoids

Flavonoids are common substances in the plant kingdom and they are usually divided into the groups of anthocyanins, chalcones, dihydroflavonols, flavonols, flavones, flavonones, flavanols, and isoflavones (Cook & Samman, 1996; Ignat et al., 2011).

The chemical structure of flavonoids shows a C6-C3-C6 configuration and they consists of 15 carbon atoms (Ignat et al., 2011). In addition to anthocyanins and flavonols, blackcurrants also contain other phenolic compounds, such as
proanthocyanins. They represent between 140 and 267 mg/100 g fresh weight in different cultivars and have been identified as e.g. catechin, (epi)gallocatechin, epicatechin, epicatechin benzylthioether and (epi)gallocatechin benzylthioether (Bakowska-Barczak & Kolodziejczyk, 2011).

The selected flavonoid compounds studied in this thesis were anthocyanins and flavonols.

2.1.1 Anthocyanins in blackcurrants

Anthocyanins are water-soluble pigments that are responsible for the red, blue and purple colour of blackcurrant fruits. The colour is pH dependent. Below pH 2 the fruit tends to present a red colour, which turns to blue and finally colourless as the pH increases (Clifford, 2000). Blackcurrants contain on average 250 mg/100 g anthocyanins (Nielsen et al., 2003), which represents about 80% of the total flavonoids. The genotype of the blackcurrant highly influences the anthocyanin content, which can vary between 80 and 476 mg/100 g fresh weight (Raudsepp et al., 2010; Nour et al., 2011).

In general, anthocyanins consist of an aglycon (anthocyanidin), sugar(s) and, in many cases, acyl group(s). Around 90% of anthocyanins are based on six anthocyanidins: pelagronidin (Pg), cyanidin (Cya), peonidin (Pn), delphinidin (Dp), petunidin (Pt) and maldivin (Mv).

The blackcurrants had all together 15 different anthocyanins, delphinidin 3-glucoside (dp-3-glu), delphinidin 3-rutinoside (dp-3-rut), cyanidin 3-glucoside (cya-3-glu) and cyanidin 3-rutinoside (cya-3-rut) being the most abundant as studied by Slimestad & Solheim (2002).

Table 3 summarizes below the major anthocyanins determined and analysed in the literature.

The existence of delphinidin compounds over the cyanidin compounds were also reported by Määttä-Riihinen et al. (2004). The amount of delphinidins were 2440 mg/kg fresh berry weight, while the amount of the cyanidins were 1452 mg/kg fresh berry weight in their study.

In the study of Landbo & Meyer (2004) the distribution delphinidin-3-rutinoside, cyanidin-3-rutinoside, delphinidin-3-glucoside and cyanidin-3-glucoside was 46-48%, 31-32%, 15-17% and 5-6%, respectively. Blackcurrant berries and juices showed the similar anthocyanin distribution as studied by Iversen (1999).
Mattila et al. (2016) investigated the anthocyanin content of different varieties of *Ribes nigrum* L. and found that rutinosides of delphinidin and cyanidin dominated, with delphinidin-3-rutinosides being the most common. Overall, the rutinosides corresponded to 74-86% and the glucosides to 14-26% of the total anthocyanins. The total anthocyanin content varied between 276±33 and 644±113 mg/100 g fresh weight for the different cultivars. However, those authors found no correlation between berry size and total amount of anthocyanins. Similarly, Milivojevic et al. (2012) found that the prevalent anthocyanin in blackcurrant was delphinidin-3-rutinoside.

Landbo & Meyer (2004) reported that the amount of anthocyanins present in freshly pressed blackcurrant juice is 1340-3220 mg/L juice, which indicates that processing into juice may contribute to loss of anthocyanins at different steps.

When juices from different countries were compared, the anthocyanin subgroups were similar to those the blackcurrant berry itself, i.e. they contained mostly dp-3-rut, followed by cya-3-rut, dp-3-glu and cya-3-glu (Mattila et al., 2011).

Veberic et al. (2015) analysed anthocyanins in blackcurrants and other wild and cultivated berries and reported the content of cyanidin, peonidin, delphinidin and petunidin to be 30.6%, 0.9%, 66.7% and 1.9%, respectively.

<table>
<thead>
<tr>
<th>Major anthocyanins</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dp-3-glu&gt;Dp-3-rut&gt;Cya-3-glu&gt;Cya-3-rut</td>
<td>Slimestad and Solheim (2002)</td>
</tr>
<tr>
<td>Dp&gt;Cya</td>
<td>Määttä-Rihinen et al. (2004)</td>
</tr>
<tr>
<td>Dp-3-rut&gt;Cya-3-rut&gt;Dp-3-glu&gt;Cya-3-glu</td>
<td>Landbo and Meyer (2004)</td>
</tr>
<tr>
<td>Dp-3-rut&gt;Cya-3-rut</td>
<td>Landbo &amp; Meyer (2004)</td>
</tr>
<tr>
<td>Major compound Dp-3-rut</td>
<td>Milivojevic et al. (2012)</td>
</tr>
<tr>
<td>Dp-3-rut&gt;Cya-3-rut&gt;Dp-3-glu&gt;Cya-3-glu</td>
<td>Mattila et al. (2011)</td>
</tr>
<tr>
<td>Dp&gt;Cya&gt;Pt&gt;Pn</td>
<td>Veberic et al. (2015)</td>
</tr>
</tbody>
</table>

Nour et al. (2013) also detected petunidin-3-rutinoside, pelargonidin-3-rutinoside, peonidin-3-rutinoside, petunidin 3-(6-coumaroyl)-glucoside, and cyanidin 3-(6-coumaroyl)-glucoside in blackcurrants, besides the four major anthocyanins described above.

Bakowska-Barczak & Kolodziejczyk (2011) identified 11 anthocyanins in different blackcurrant cultivars. In addition the anthocyanins already reported in this chapter, they also identified peonidin-3-glucoside and petunidin-3-glucoside,
with an average content of 0.64-2.46 and 0.13-2.54 mg/100 g fresh weight, respectively. The structures of common anthocyanins are illustrated in Figure 2.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyanidin-3-O-glucoside</td>
<td>H</td>
<td>O-glucose</td>
<td>OH</td>
</tr>
<tr>
<td>Cyanidin-3-O-rutinoside</td>
<td>H</td>
<td>O-rutinoside</td>
<td>OH</td>
</tr>
<tr>
<td>Delphinidin-3-O-glucoside</td>
<td>OH</td>
<td>O-glucose</td>
<td>OH</td>
</tr>
<tr>
<td>Delphinidin-3-O-rutinoside</td>
<td>OH</td>
<td>O-rutinoside</td>
<td>OH</td>
</tr>
</tbody>
</table>

Fig. 2. Structure of common anthocyanins (© Vagiri 2012).

### 2.1.2 Flavonols in blackcurrants

The amount of flavonols in berries depends strongly on environmental factors such as moisture, light, etc. the conditions that are naturally available during the cultivation period. It has been concluded that flavonols are still bioavailable in berries grown in shade (Šavikin et al., 2013).

Significant differences have been found regarding the flavonol content of different blackcurrant cultivars, e.g. the total content of flavonols is reported to be between 9.6 and 21.6 mg/100 g fresh weight, with myricetin, quercetin and kaempferol being the most abundant flavonol aglycons (Mattila et al., 2016). Those authors also found a correlation between the anthocyanin and flavonol contents, which was positive for quercetin and anthocyanin cyanides and negative for anthocyanin delphinides. For myricetin, a positive correlation was found for delphinindins and a negative correlation for cyanidins. There was no correlation between the flavonol content and berry size (Mattila et al., 2016).

Studies show that the flavonols vary among the different blackcurrant cultivars (Häkkinen et al., 1999a; Mikkonen et al., 2001). The main flavonol in these berries is myricetin or quercetin, followed by kaempferol. Different data exists of the amounts of these compounds in the blackcurrant berries. In the study of Häkkinen et al. (1999b) 5.5 mg/100 g myricetin and 3.3-6.8 mg/100 g quercetin of fresh weight was reported. In the work of Määttä-Riihinen et al. (2004) the flavonol glycosides were reported and myricetin comprised 98 mg/kg, quercetin 50 mg/kg and kaempferol 13 mg/kg of fresh weight. The chemical structure of the most common flavonols, myricetin, quercetin, kaempferol and isorhamnetin is illustrated in Figure 3.
Fig. 3. Chemical structure of common flavonols (© Vagiri 2012).

Opposite to this, in the work of Herrmann (1976) the composition of the blackcurrant variety ‘Silvergieters Schwarze’ showed the domination of quercetin, followed by kaempferol. In his study, myricetin was not analysed.

Aglycons of kaempferol, myricetin and quercetin have been detected in blackcurrant berries by Milivojevic et al. (2012), while Gavrilova et al. (2011) only observed the presence of rutinosides of myricetin and quercetin.

When the flavonol structure of blackcurrant juices has been investigated, it was concluded that juices from different countries vary greatly in terms of their flavonol content. A 12-fold variation in flavonol content was observed by Mattila et al. (2011), with values ranging between 0.6 mg and 7.6 mg/100 mL juice. In addition, the flavonol composition was found to be similar to that of the blackcurrant berry, containing mostly rutinoside, galactoside and glucoside derivatives of myricetin, quercetin and kaempferol (Mattila et al., 2011).

Bakowska-Barczak & Kolodziejczyk (2011) reported the conjugates of glucoside and rutinoside of myricetin, quercetin and kaempferol. The berries contained 1.9-11.2 mg/100 g fresh weight myricetin derivatives, 1.3-4.7 mg/100 g fresh weight quercetin derivatives and 0.7-1.9 mg/100 g fresh weight kaempferol derivatives.

2.2 Effect of processing on flavonoids

Conventional juice processing involves many processing steps, such as crushing, heating, enzyme maceration, pressing, filtration, clarification, pasteurisation, bottling and storage (Mäkilä et al., 2016).

Depending on the processing and storage methods applied, the proportion of flavonoid compounds in the final product may be reduced, due to their sensitivity. It has been reported that, during heat treatment, the anthocyanin content decreases
in grape and strawberry juices (Bakker & Bridle, 1992; Garzon & Wrolstad, 2002; Talcott et al., 2003). The heating process has a significant effect on the flavonoid content of different food matrices, showing decrease in their concentration (Ioannou et al., 2012).

Clarification of juices on an industrial scale usually involves gelatin-silica sol treatment, vacuum filtration and other filtration methods. It has been shown that the gelatin-silica sol treatment followed by vacuum filtration has a significant effect on the total phenol level and the content of four major anthocyanins in blackcurrant juice, with a reduction of 21% in total phenols and 19-29% in anthocyanins (Meyer & Bagger-Jørgensen, 2002). A decrease in anthocyanins was also observed by Patras et al. (2011), who found that the content varied between 13.2 and 37.1% during storage of a strawberry jam when the storage conditions were 7 days at 4 °C and 28 days at 15 °C, respectively. Thus, it is of critical importance to design the treatment process so that valuable compounds are preserved until the end of life of the product.

Besides anthocyanins, the other main flavonoid compounds investigated in this thesis, the flavonols, are sensitive to high temperatures as well. A sharp decrease in flavonol content has been observed at temperatures higher than 45 °C (Cacace & Mazza, 2003).

The processing and storage of the berries was studied by Häkkinen et al. (2000) to follow how the flavonol content is affected. Blackcurrants are usually processed in Finland into juice using steam extraction, although research results indicated that the flavonols are better preserved if cold-pressing was used. In the latter processing method 45% of the quercetin and 65% of the myricetin was found in the blackcurrant juice, while in the steam extraction process 15% and 30%, respectively.

For the loss of the anthocyanins during the juice processing the main reasons can be the enzymatic breakdown and the removal of these compounds with the pulp. Also the storage affects significantly the anthocyanin content; a 50% decrease in the monomeric anthocyanin content was measured when the juices were stored for 6 months in daylight at 20 °C (Iversen, 1999).

Anthocyanins have been found to be sensitive to higher processing temperatures; between 25 and 30 % of the anthocyanins were lost, and the heat treatment at 98 °C was found to be the most significant reason for this loss (Mikkelson & Poll, 2002). Gössinger et al. (2009) reported on the decrease of anthocyanins in strawberry nectar from puree when pasteurization at 85 °C for 10 minutes was applied.
The effect of enzymatic treatment which is generally applied in juice processing for increasing the juice yield also has an effect on the anthocyanins distribution. Buchert et al. (2005) investigated numerous enzyme preparations for the treatment of blackcurrant berries. The treatment was carried out at 45 °C for two hours, and Econase CE, Pectinex Smash, Pectinex BE-3L, Pectinex Ultra SP-L or Biopectinase CCM was dosed corresponding to 1000 nkat/g. The authors reported an increase in the relative content of delphinidin and cyanidin glucosides from 21% to 26-37% in the pectinase-treated juice. At the same time, the rutinosides decreased from 79% to approximately 63-74%.

In contrast, Landbo & Meyer (2004) reported no change in the relative distribution of the four major anthocyanins during the enzymatic maceration process of the berries when they made an extensive study of different enzymes corresponding to 250 experimental runs. The enzymes used were Macer 8, Pectinex Superpress, Pectinex BE, Pectinex Ultra SP-L, Rapidase BE Super, Rohapect B5L, Rapidase EX Color, Klerzyme Color, Rapidase Vino Super, and Vinozyme G at an E/S ratio of 0.05% using processing conditions of 7, 30, 50 and 70 °C with a holding time of 10, 60, 90, 180 and 300 minutes. Enzyme-assisted extraction of blackcurrant juice significantly increased the content of anthocyanins and other phenolic compounds in the blackcurrant juice when Macer and Biopectinase enzymes were tested in 12 and 1200 nkat/g concentration and were held either at 40 ± 2 °C or 50 ± 12 °C for two hours (Laaksonen et al., 2012).

Furthermore, the juice yield increased by approximately 10-22% when Pectinase 714L was used with a dose of 21.7 U and hydrolysed at 45–47 °C for four hours (Laaksonen et al., 2013), compared with a non-enzymatic juice extraction. The amount of total phenolic compounds, including anthocyanins, flavonols and hydroxycinnamic acid, increased by 4- to 10-fold in the case of enzyme-treated berry juices (Laaksonen et al., 2013).

In the study of Mieszczakowska-Frąc et al. (2012), the blackcurrant juice yield increased from 62 g/100 g to 73 g/100 g and from 65 g/100 g to 75 g/100 g after applying P.BE.C. and P.BE.XXL enzyme preparations in a dose of 100, 200 and 400 g/t during the treatment for four hours at 50 °C, respectively. When observing the changes in the anthocyanin content, it was concluded that a prolonged enzyme treatment time (from 1 to 4 hours) decreases the amount of anthocyanins and increases the loss of anthocyanins (between 14.8-25.1%). The amount of enzyme used in the maceration process does not influence the anthocyanin content of the juices.
In the juice manufacturing process, the marc left behind contains high amounts of phenolic antioxidants, especially anthocyanins, and the anthocyanin composition of the seedless marc is similar to that of the blackcurrant fruit itself (Kapasakalidis et al., 2006).

Anttonen & Karjalainen (2006) reported that marc also contains myricetin glucosides (glu, rut, mal) and three quercetin glucosides (glu, rut, mal). They also detected traces of kaempferol glucoside. Šojka & Król (2009) separated the dried marc into three different size fractions (<0.8 mm, 2-5 mm and >5 mm) and observed that the amount of polyphenols is highly dependent on the size class being the most abundant in the fraction of 2-5 mm.

Anthocyanins show first-order degradation reaction kinetics in a conventional heating process, and it was observed that if the temperature is elevated, the degradation would increase (Verbeyst et al., 2010). The operation parameters used in the experiments were the temperature range between 80-130 °C and the pressure range of 200-700 MPa. In addition, the study investigated the combined effect of elevated pressure and temperature, and showed that the degradation of anthocyanins followed the first-order kinetics, but temperature having a more profound effect than pressure.

High pressure treatment of strawberry anthocyanins was also investigated by Zabetakis et al. (2000). The processing was carried out at 200, 400, 600 and 800 MPa for 15 minutes at temperatures between 18-22 °C. It was concluded that anthocyanins were preserved in the treatment, but were more susceptible to degradation during storage if the temperature was increased from the refrigeration temperature to 20 and 30 °C. This phenomenon can be attributed to the effect of high pressure on the enzyme activity involved in the degradation process.

Clarification is often a crucial step in juice processing to remove the compounds that cause haze in the juice. It was shown that clarification has a significant effect in the decrease of the anthocyanins in pomegranate when gelatin at a level of 0.5% and 1% (w/v%) was used at 5 °C for treatment (Turfan et al., 2011). The results indicated that the decrease was 4% in the case of the pomegranate juice from “juice sacs”, and 19% in the case of “juice from the whole fruit”.

High temperature, short time treatment of blackcurrant juice has been shown to be superior to low temperature, long time treatment in terms of loss of anthocyanins (Mikkelsen & Poll, 2002). The authors reported a loss of 25-30% of anthocyanins during the whole processing which included enzyme treatment with pectinases, pasteurization at 98 °C either for 30 or 60 seconds, clarification by
gelatin and kieselsol, and filtration through 6.0 and 4.3 mm mesh size filters. Although clarification also affected, the more profound effect was attributed to the heat treatments causing the loss of anthocyanins. Pasteurisation at 95 °C for 10 minutes has been found to result in the loss of 8% and 13% of anthocyanins in pomegranate juice made from the sacs and the whole fruit, respectively (Turfan et al., 2011). The degradation of the anthocyanins is also influenced by the presence of polyphenol oxidase enzyme (Patras et al., 2010). The polyphenol oxidase activity can be terminated by mild heat treatment for example at 60 °C ± 1°C (Wang & Xu, 2007), which can thus have a positive effect on the anthocyanin preservation.

Storage conditions may also cause the loss of anthocyanins. Brownmiller et al. (2008) reported the loss of almost 40% of anthocyanins in blueberry products (puree, canned fruit and juice) after 6 months of storage at 25 °C, while Hager et al. (2008) observed 65.8% loss in the anthocyanins in the case of canned-in-syrup over a similar storage period of 6 months at 25 °C.

Capanoglu et al. (2013) observed almost complete destruction of delphinidin-3-glucoside, i.e. 99% of these compounds were destroyed, when processing grape berry into grape juice concentrate. The processing steps were pre-heating to 40-50 °C, mashing, pressing, pasteurization at 100-107 °C, clarification including enzyme treatment (pectinase and amylase), removal of suspended solids and fining, followed by filtration by diatomaceous earth filter and concentration by evaporation. The 3-glucoside of cyanidin, peonidin and malvidin decreased by 94, 95 and 98%, respectively. Different processing steps were shown to have selective effects on two anthocyanin types, delphinidin-3-glucoside and acylated anthocyanins, which were reduced more intensively in the pasteurisation step, while other anthocyanins were mostly reduced in the clarification and filtration steps. In addition to anthocyanins, a sharp decrease in flavonols was observed, with only 50% of the original flavonol content preserved in the grape juice concentrate.

In blackcurrant juice production, the loss of total anthocyanin of 75% (from 364 ± 8.0 mg/100 g to 87.4±2.8 mg/100g in pasteurized nectar) was reports in juice processing by Iversen (1999) when the processing of the juice included mash treatment by Pectinex AR in a concentration of 0.0057% at 50 °C for two hours. After enzyme treatment, the mash was centrifuged at the same temperature, pasteurized at 87 °C for 27 second, followed by bottling. The loss of anthocyanins was mainly attributed to the pulp removal step by centrifugation. On the other
hand, a significant loss also occurred during the storage for 6 months at 20 °C either in dark or in daylight.

Cavalcanti et al. (2011) made an extensive review article on the factors affecting anthocyanin stability, and concluded that pH plays the most important role in anthocyanin degradation, compared with temperature, concentration, oxygen, light, enzymes, ascorbic acid, sugars and sulphites.

Rein (2005) described that in general anthocyanins are more stable at low acidic pH than in alkaline solutions. The pH of the solution will determine in which one of the four species it will appear: at highly acidic conditions it will show the flavylum cation with a main color of red. If pH is increased, the quinonoidal base will be dominant by blue and violet colour. This is followed by the formation of the carbinol or pseudobase and the colourless chalcone species.

Askar, Alsawad & Khalaf (2015) reported that anthocyanins of rosella extracts undergo a degradation process quickly during storage and processing. The authors observed that the higher the pH was, the greater the extent of the degradation was found. When compared at pH 9 after 180 minutes to pH 1, they described that the degradation at high pH was about 20% of total anthocyanins, while at low pH, the extent of degradation was found to be only 6% when the conditions were dark and storage at room temperature. From anthocyanidins, cyanidin and delphinidin, studied also in this thesis, are stable in acidic conditions as reviewed by Khoo et al. (2017) without giving an emphasis on other environmental factors in their study.

Hellström et al. (2013) reported a significant effect of temperature on the anthocyanin degradation rate, with the degradation being fastest at room temperature (21 ºC), followed by 9 ºC and 4 ºC storage temperature.

Overall, to ensure the stability of the anthocyanins, there is a need for a complete understanding of the complex process, with all factors affecting it to ensure that they are not degrading during the processing and storage.

2.3 Major health effects of blackcurrants

In addition to the nutritive value, blackcurrants are also consumed due to their potential health benefits. Their protective actions are exerted on different parts of the human body, such as the cardiovascular system, nervous system, ocular system, skeletal system and renal system, and they play a role in wound recovery through their healing properties (Gopalan et al., 2012).
Benn et al. (2014) investigated the anti-inflammatory effect of blackcurrant extract on adipose tissue and splenocytes and concluded that blackcurrant consumption decreases obesity-induced inflammation by enhancing energy metabolism in skeletal muscles.

Anthocyanins show potential in cancer prevention due to their anticarcinogenic properties. Bishayee et al. (2010) and Bishayee et al. (2011) used a blackcurrant skin extract to study inhibition of HepG2 liver cancer cells and chemically induced hepatocarinogenesis in rats, respectively, and confirmed the inhibitory effects.

Furthermore, it has been reported that blackcurrant can reduce the proliferation of different cancer cells, such as breast and colon cancer. Jia et al. (2012) reported good effectiveness of blackcurrant in inhibition of gastric cancer by inducing cell apoptosis, thus indicating the potential of these berries as therapeutic agents in gastric cancer treatment.
3 Use of pressure-driven membrane processes in juice processing

In pressure-driven membrane processes, the separation takes place due to a pressure difference between the two sides of the membrane. Usually, for fruit juice processing, integrated membrane techniques are developed which would include different operations connected in series, such as the combination of micro/ultra/nanofiltration or reverse osmosis, with the final concentration step with membrane distillation and aroma recovery by pervaporation (Sotoft et al., 2012).

These processes are low-cost techniques when compared with that of the traditional juice processing techniques that involves the cost-intensive pre-treatment of the juice such as enzyme treatment, pre-filtration supported by the use of filtration aids as a part of the clarification, followed by thermal evaporation.

Sotoft et al. (2012) analysed that if the production of blackcurrant juice concentrate is based on the combination of membrane technologies (vacuum membrane distillation combined with a nanofiltration/reverse osmosis step, and the final concentration with direct contact membrane distillation), the final juice concentrate can be produced with a sugar content of approximately 65-70%. With the implementation of these techniques, the operation cost of an industrial plant can be reduced by 43% in blackcurrant juice concentrate production when compared to a traditional evaporation process, and the overall economy of the membrane-based processing is at least competitive with the traditional process including the evaluation of investment and labour costs.

Since in membrane-based concentration processes, the clarification and the concentration take place at much lower temperatures than are typically used in concentration by evaporation, the phase change in the juice will be avoided (Cassano et al., 2010). Kozak et al. (2008) developed an integrated membrane process for the concentration of blackcurrant juice in which reverse osmosis was used for the pre-concentration of the sample at 20 °C and 51 bar achieving 26 °Brix at the end of the process.

In traditional juice processing, for concentration purposes, a multi-stage vacuum evaporation process would be used. This process, even if carried out under vacuum, will require the application of higher temperatures than in reverse osmosis which will have in turn an effect on the quality of juice in terms of a loss in valuable compounds and by developing the cooked taste of the juices.
Elik et al. (2016) studied the feasibility of a rotary vacuum evaporator for the concentration of blueberry juice, but even in this process, the temperature applied reaches 45 °C, while the final total soluble solid content of 65 °Brix is achieved.

In general, in membrane processes, one or more compounds are selectively permeable through the membrane when a hydrostatic pressure gradient is applied (Echavarria et al., 2012). The membrane itself is a “selective barrier between two phases, the term ‘selective’ being inherent to a membrane or membrane process” (Mulder, 1991).

Pressure-driven membrane processes can be divided into four classes; microfiltration, ultrafiltration, nanofiltration and reverse osmosis. These processes differ from each other in terms of membrane pore size and the transmembrane pressure applied, which together determine which compounds will be separated. A summary is given in Figure 4. The membrane pore size in microfiltration is between 0.1-10 μm and the operating pressure is usually lower than 2 bar. In ultrafiltration, the pore size is 0.01-0.1 μm and the pressure range is 1-10 bar. The nanofiltration process uses a membrane pore size of 1-10 nm and the reverse osmosis a membrane pore size of 0.1-1 nm. However, in these two processes the applied pressure is higher, 10-25 bar in nanofiltration and 15-80 bar in reverse osmosis (Mulder, 1991).
In microfiltration and ultrafiltration the separation principle is based on a sieving mechanism, while in nanofiltration and reverse osmosis the separation can be described by a solution-diffusion model.

The membrane materials also vary between these processes. In microfiltration and ultrafiltration, polymeric and ceramic membranes are usually used. In practice, the membranes are prepared from polymeric materials through phase inversion, being polysulphon/polyethersulphone, polyvinylidene fluoride or polyetherketone as examples in MF and UF processes. In addition, inorganic materials such as zirconium- or aluminium oxide are used as ceramic membranes in these processes.

On the other hand, in reverse osmosis, the polymeric materials for asymmetric membranes are usually cellulose esters, polyamides, or polybenzimidazoles and polyimides. In the case of composite membranes, in which the top layer and the sublayer are composed of different polymeric
materials, the material used for NF are different polyamides, and cellulose triacetate, aromatic polyamide or polyamide materials in RO (Mulder, 1991).

Microfiltration, ultrafiltration, and nanofiltration and reverse osmosis were successfully implemented in the clarification, stabilisation, depectinisation and concentration of fruit juices (Báñovölgyi et al., 2009; Cassano et al., 2010; Yazdanshenas et al., 2010; de Oliveira et al., 2012; Sotoft et al., 2012).

Membranes as a concentration method have several advantages over the conventional evaporation concentration method. The disadvantages of evaporation at high temperatures include loss of aroma and nutrients (Calabro et al., 1994; Varming et al., 2004; Alves & Coelho, 2006), and the induction of cooked odour (Cross, 1989; Calabro et al., 1994), and the large amount of energy is needed. In addition, complex membrane-based concentration, including microfiltration, reverse osmosis and nanofiltration, of the grape juice has been found to be much more economically feasible than the conventional evaporation process (Kiss et al., 2004).

3.1 Performance of pressure-driven membrane processes

In this section, major factors affecting membrane processes, such as transmembrane pressure, pure water flux, volumetric reduction factor and retention of the membrane, are introduced.

The transmembrane pressure (ΔP_{TM}) is defined as:

\[ \Delta P_{TM} = \left( \frac{P_{in} + P_{out}}{2} \right) - P_{perm} \text{(bar)} \] (1)

where \( P_{in} \) and \( P_{out} \) are the inlet and outlet pressure of the membrane module (bar), and \( P_{perm} \) is the pressure at the permeate side (bar).

The pure water flux is expressed as:

\[ J_w = \frac{\Delta P_{TM}}{\eta_w R_M} \text{(L/m}^2\text{/h)} \] (2)

where \( J_w \) is the pure water flux (L/m\(^2\)/h), \( \Delta P_{TM} \) is the transmembrane pressure (bar), \( \eta_w \) is the water viscosity at 20 °C (Pas), and \( R_M \) is the membrane resistance (/m).
The volumetric reduction factor is determined using the following equation:

\[
VRF = \frac{V_0}{V_R} = \frac{V_0}{V_0 - V_P}
\]  \hspace{1cm} (3)

where \(V_0\) is the volume of the feed (L), \(V_R\) is the volume of the retentate (L) and \(V_P\) is the volume of the permeate (L).

The retention of the membrane is determined for the total soluble solids content and the anthocyanin and flavonol content, and expressed as a percentage using the following equation:

\[
R = \frac{c_R - c_P}{c_R} \cdot 100 \left(1 - \frac{c_P}{c_R}\right) \cdot 100 \% \hspace{1cm} (4)
\]

where \(R\) is the retention of the membrane (%), \(c_R\) is the concentration of the desired compound in the retentate (g/L) and \(c_P\) is the concentration of the desired compound in the permeate (g/L).

### 3.2 Concentration polarisation

The initial decrease in the permeate flux is generally explained by the effect of concentration polarisation, while the second phase of the decrease is due to accumulation of molecules and particles on the membrane surface or inside the pores of the membrane as the concentration of the feed solution increases (Condini et al., 2015).

It is important to make a distinction between concentration polarisation and fouling of the membrane. Although fouling is often the consequence of the concentration polarisation, it will be caused by the actual deposition of the feed material constituents such as particles, macromolecules, salt, etc. on the membrane surface or inside the membrane (Mulder, 1991).

Concentration polarisation occurs when the solute concentration gradually increases in the membrane boundary region. This concentration build-up will cause a diffusive flow back to the bulk of feed, as described by Mulder (1991) and illustrated in Figure 5. This diffusive flow backwards will be followed by the establishment of the steady-state condition.
Concentration polarization as a phenomenon is reversible in its nature, and it will cease once the pressure is released in the process (Aimar et al., 1994). There is also possibility to reduce the magnitude of it by the manipulation of flow velocity in the membrane (Ochando-Pulido et al., 2015), or by changing the module configuration (Mulder, 1991).

Due to concentration polarization, an additional resistance occurs that raises the operating costs and negatively affects the permeate quality (Ochando-Pulido et al., 2015). As a result of the concentration polarization, the retention of the membrane can change (Mulder, 1991). Furthermore, it will cause the permeate flux to decrease, which, however, reaches steady-state conditions after quite a short period.

### 3.3 Fouling of membranes

The term membrane fouling is often used to describe a long-term decline in permeate flux caused by the accumulation of some compounds on the membrane surface, for the reason of concentration polarization, gel layer formation or blockage of the membrane pores (Mulder, 1991).

The fouling mechanism usually involves either pore blocking, plugging or clogging and cake formation on the membrane surface (Ochando-Pulido et al., 2015), or the adsorption of the compounds either on the surface or within the
pores of the membrane, and will result in an additional adsorption resistance (Mulder, 1991).

The foulant can be organic precipitates (e.g. macromolecules), inorganic precipitates (e.g. salts) or particulate material. Fouling always results in a capital loss, due to the decrease in the permeate flux, membrane selectivity and shutdowns to clean the membranes, and shortening of the membrane lifetime. The membrane fouling can be reversible or irreversible. If the fouling is irreversible, physical or chemical cleaning does not help to recover the membrane and it has to be replaced.

To this end, it is very important to design the membrane processes so that fouling problems can be avoided or at least minimized. One of the many possibilities for this purpose is pre-treatment of the feed. This can sometimes involve very simple methods such as pre-filtration or clarification of juices. Selection of an appropriate membrane material may also help in reducing fouling of the membranes. As an example, the use of hydrophilic membranes instead of hydrophobic membranes in the case of protein filtration can help to avoid severe fouling.

In addition to the pre-treatment of the feed, and the careful selection of the membrane properties, also the optimization of the operation conditions, e.g. by adjusting high flow velocities and the application of turbulence promoters may help to reduce or eliminate fouling in the membrane filtration process. However, most often there is a need for cleaning procedures when fouling of the membrane occurs. Cleaning might be either chemical, mechanical, electric or hydraulic cleaning.

One method to reduce fouling of the membrane materials is the back-flushing procedure (Mulder, 1991). In this hydraulic cleaning approach, after a finite time of filtration, the direction of the flux is reversed from the permeate side to the feed side by removing the pressure at the feed side, in order to remove the fouling layer that has formed. The disadvantage of this technique is that it can be applied only in certain membrane filtration applications, mostly in microfiltration and to some extent in ultrafiltration.

However, the most frequently used method to reduce fouling still remains the chemical cleaning of the membranes. To be successful, the operating conditions and the concentration of the cleaning agent should be well defined. The cleaning materials are usually acids, alkali, detergents, enzymes, complexing agents, disinfectants, steam and gas (Mulder, 1991).
3.4 Mathematical modelling of membrane processes

There are a number of models that can describe the flux decline in membrane filtration, and they are usually categorised into osmotic-pressure-controlled, gel polarisation and resistance-in-series models (Purkait et al., 2004).

3.4.1 Resistance-in-series model

Resistance-in-series modelling has been applied for instance to ultrafiltration of kiwifruit juice (Cassano et al., 2007), apple juice (Vladiesavljević et al., 2003), passionfruit juice (Jiraratananon & Chanachai, 1996), mosambi juice (Rai et al., 2006, 2007) and pomegranate juice (Bagci, 2014).

Bagci (2014) described the resistance-in-series model based on Darcy’s law as follows:

\[ J = \frac{\Delta P}{\mu R_T} = \frac{\Delta P}{\mu(R_M + R_C + R_F)} \quad (\text{L/m}^2/\text{h}) \quad (5) \]

where \( \Delta P \) is the transmembrane pressure (bar), \( \mu \) is the viscosity of the permeate (Pas), \( R_M \) is the membrane resistance (/m), \( R_C \) is the cake layer resistance (/m) and \( R_F \) is the fouling resistance (/m).

The resistance model of membrane separation defines the pure water flux as the quotient of the trans-membrane pressure, i.e. the driving force \( \Delta P_{TM} \) (Pa), and the membrane resistance \( R_M (/m) \), calculated by water dynamic viscosity \( \eta_W \) (Pas) arising from the pore size of the membrane.

\[ J_W = \frac{\Delta P_{TM}}{\eta_W \cdot R_M} \quad (\text{L/m}^2/\text{h}) \quad (6) \]

The fouling resistance of the membrane used can be determined from the water flux \( J_F \) measured at a fixed temperature after flushing the membrane with tap water after the concentration of the juice was carried out in the batch concentration mode to a desired °Brix value, using the following formula:

\[ R_F = \frac{\Delta P_{TM}}{J_F \cdot \eta_W} - R_M \quad (/m) \quad (7) \]
The total resistance is composed of three resistances:

$$R_t = R_M + R_F + R_p$$  \hspace{1cm} (/m)  \hspace{1cm} (8)$$

where $R_p$ is the polarisation layer resistance.

The total resistance ($R_t$) can be calculated from the permeate flux value at the end of the concentration using the following formula:

$$R_t = \frac{\Delta P_{TM}}{\eta_p J_p}$$  \hspace{1cm} (/m)  \hspace{1cm} (9)$$

where $\eta_p$ is the juice viscosity (Pas) at the operation temperature of the filtration, and $J_p$ (L/m$^2$/h) is the juice permeate flux at the end of the filtration.

Once the values of the membrane resistance, fouling resistance and the total resistances are determined, the polarization layer resistance can be calculated using the equation (8).

In general, the resistance-in-series model is empirical in its nature. The model is not based on predicting the actual phenomena and the model parameters calculated by the experiments lack physical meaning. The experimental values obtained during the concentration tests are compared with the calculated values obtained by the model. Thus, the model lacks flexibility and generality from the point of view of prediction of permeate flux decrease for different types of fruit juice materials.

Therefore, the profound understanding of the theory behind the behaviour of the fruit juice is relevant. Especially, when carrying out experiments in the batch concentration mode, the real phenomena, e.g. the time variation of the different characteristics, such as the viscosity, gel formation, etc. have to be taken into account when carrying out the modelling of the process by the utilization of the resistance-in-series model (Mondal et al., 2011).

### 3.4.2 Osmotic pressure and modelling

The dissolved molecules of the feed material in the boundary layer of the membrane play a role in the creation of osmotic pressure. The osmotic pressure is the minimum pressure to be applied in order to prevent the flow of water across the semi-permeable membrane in the separation process of two solutions in order to stop the osmosis process.
To achieve permeation through the membrane in the reverse osmosis process, this osmotic pressure should be determined and overcome by the application of an external pressure. The osmotic pressure is of greater importance in the process of reverse osmosis, and much less relevant in other pressure-driven membrane processes such as microfiltration and ultrafiltration (Mulder, 1991).

To determine the osmotic pressure, the van’t Hoff model can be applied, which describes the osmotic pressure dependence on the difference in concentrate \( c_R \) (kmol/m\(^3\)) and permeate \( c_P \) (kmol/m\(^3\)) molar concentration by the following formula:

\[
\Delta \pi = (c_R - c_P) \cdot R \cdot T \quad \text{(Pa)}
\]

(10)

where \( R=8314.472 \) J/kmol/K is the universal gas constant, \( T=298.15 \) K is the temperature of experiment. As the formula shows, the osmotic pressure is dependent on the molar concentration of the solute, and not by the type of the solute. Therefore if during the concentration process, the solute dissociates or associates, the number of moles will change, increases in dissociation and decreases in association, which will increase and reduce the osmotic pressure, respectively (Mulder, 1991).

In the reverse osmosis process, the solute concentration of the permeate side \( c_P \) is usually very low, sometimes even two orders of magnitude lower than in the retentate side. Therefore in this thesis, the permeate side solute concentration is neglected and the concentration polarisation \( \beta=\frac{c_M}{c_R} \), where \( c_M \) is the concentration of the compound in (kg/m\(^3\)) at the membrane surface is introduced into the previous equation and it gives the following formula:

\[
\Delta \pi = \beta \cdot c_R \cdot R \cdot T \quad \text{(Pa)}
\]

(11)

By combining the equations (10) and (11), the following equation is obtained for determining the permeate flux during the filtration:

\[
J = \frac{\Delta P_M}{\eta W R_T} - \frac{\beta R T}{\eta W R_T} \cdot c_R \quad \text{(L/m\(^2\)/h)}
\]

(12)

On plotting the permeate flux against \( c_R \), from the intercept of the fitted straight line the average values of the total resistances during concentration of blackcurrant juice can be calculated.
3.5 Clarification of fruit juices by membrane technology

Microfiltration

In the microfiltration of cactus pear juice, Cassano et al. (2010) observed that the flux decline could be divided into three parts when the permeate flux decreased gradually: the initial period in which a sharp decline in the permeate flux occurs, the second stage in which a smaller decrease in the flux is observed and the third phase in which the permeate flux achieves steady state in the microfiltration experiments when polyvinylidenfluoride (PVDF) flat sheet membranes were used with a pore size of 0.20 μm at a transmembrane pressure of 2.2 bar, feed flow rate of 500 L/h and a processing temperature of 25 °C.

This phenomenon during the filtration process is described by the concentration polarization and gel layer formation on the membrane surface. The microfiltration process was successful in terms of the quality of the juice, since it did not alter the total polyphenol content of the juice.

Similar observations were made also in ultrafiltration of cactus pear juice using the same operation parameters, and a PVDF membrane with a cut-off value of 200 kDa (Cassano et al., 2010). The main properties of the cactus pear juice were not altered also in the ultrafiltration process, but the suspended solids were completely removed ensuring the clarification of the juice samples.

Laorko et al. (2010) investigated the microfiltration of pineapple juice, with particular regard to the membrane pore size and the corresponding permeate flux. They observed that in a hollow fiber module, the flux of a 0.2 μm polysulphone membrane was not significantly higher than that of a 0.1 μm polysulphone membrane and attributed this to the pore blocking effect of the juice constituents, which more easily blocked the larger pores in the membrane. Those authors also investigated the effect of operating conditions on the permeate flux. On changing the transmembrane pressure (TMP), higher fluxes were achieved at the lower TMP, while when TMP was increased a critical flux was reached at 0.7 bar pressure.

The cross-flow velocity also had an effect on the permeate flux, where the higher the cross-flow velocity, the higher the permeate flux (Laorko et al., 2010). This is due to the fact that the shear stress on the membrane surface is increased and this affects the concentration polarisation and the membrane fouling.
Ultrafiltration

Juices and other feed materials usually contain pectin in high amounts, proteins and other carbohydrates (Rai et al., 2007). Ultrafiltration is a viable method to remove of microorganisms and pectin from the juices, and therefore ensure the microbiological quality of the end-product, but also ensure that the haze formation in the case of the juices is avoided, and therefore the filtration process is more effective in terms of permeate flux as the fouling of the membrane can be minimized.

Cissé et al. (2011) described that the evolution of permeate flux during ultrafiltration is a more complex process, and is not only dependent on the transmembrane pressure, but also on membrane properties such as the material or structure when the performance of a thin-film membrane with 1 kDa MWCO and a polyethersulfone membrane with 50 kDa was studied at 2 MPa operation pressure at temperature of 35 ± 0.5 °C. They described that also the interactions between the membrane and the solute does have an effect on the permeate flux in ultrafiltration of the roselle extracts (Hibiscus sabdariffa L.).

Destani et al. (2013) applied 100 kDa hollow fibre ultrafiltration membrane made of polysulphone for clarification of blood orange juice in a batch concentration mode prior to concentration using operation conditions of transmembrane pressure of 0.8 bar, an axial feed flow rate of 1760 mL/min and a temperature of 20 ± 1 °C. The experiments were run until a weight reduction factor of 3.8 was reached. They observed a gradual decrease in the permeate flux in the ultrafiltration process, due to accumulation of juice components in the pores of the membrane (fouling) and on the membrane surface (concentration polarisation and gel layer formation). The permeate flux decreased from an initial 14.02 kg/m²/h to about 2.7 kg/m²/h.

A similar effect was observed when depectinised bergamot juice was clarified in ultrafiltration process. The initial flux of 9 kg/m²/h declined sharply in the beginning of the process, by 50% in the first 15 minute when a hollow fiber module equipped with polysulphone 100 kDa NWCO membrane was used under the operation conditions of a transmembrane pressure of 0.7 bar, an axial feed flow rate of 114 L/h and a temperature of 24 °C were used. The concentration was carried out according to the batch concentration mode until the volume reduction factor of 7.8 was reached (Condini et al., 2011). The authors also observed a limiting flux while ultrafiltering the clarified bergamot juice using a flat sheet configuration including a fluoropolymer 1000 kDa membrane at the operation
conditions of transmembrane pressure of 7.5 bar, an axial feed flow rate of 97.6 L/h and a temperature of 24 °C.

The pH of the juice can also affect the performance of ultrafiltration. It has been observed that on increasing the pH to 8.5, a reduction in the permeate flux occurs, which was attributed to changes in the chemical composition of the juice, especially precipitation of polyphenols at higher pH (Chethan & Malleshi, 2007), which may play a role in the fouling of the membranes.

Different pre-treatments may result in an increase in the permeate flux. Bagci (2014) studied the effect of gelatin, gelatin and bentonite, polyvinyl polypyrrolidone (PVPP), and PVPP combined with bentonite treatments before ultrafiltration. The authors observed a significant increase in the permeate flux in the case of the filtration after the gelatin, and PVPP combined with bentonite treatments when using a 30 kDa NWC polyvinylidenfluoride membrane at a filtration temperature of 25 °C, transmembrane pressure of 3 bar and a recirculation flow rate of 700 L/h. On the other hand, the PVPP-treated juice showed higher fluxes than the gelatin-treated.

Pre-treatment of the juice may prevent fouling of the ultrafiltration membranes, as observed by de Bruijn et al. (2003). They investigated the effect of a combined enzymatic treatment with bentonite, gelatin and activated carbon, followed by filtration through a Whatman no. 42 filter on the permeate flux during ultrafiltration. In ultrafiltration, Carbosep membranes with a nominal MWCO of 15 and 50 kDa were used made of zirconium dioxide with a porous carbon support. The operational parameters were temperature of 50-55 °C, pressure of 150 or 400 kPa and velocity of 2 or 7 m/s. Due to the pre-treatment, the initial permeate flux decreased sharply by 45% in the first 5 minutes of the experiment, followed by a slow flux decline. This decrease in flux can be attributed to adsorption of compounds on membrane surface, gel layer formation and pore blocking, as reported by the authors.

Since the disadvantage of the rapid reduction in the permeate flux during ultrafiltration is that this phenomenon restricts the applicability of the process at commercial level in the clarification of fruit juices (Onsekizoglu, 2013; Domingues et al., 2014), an effort has to be made to understand and model the mechanism behind the flux decrease, and improve the process performance so that it can be implemented economically in juice production facilities.
3.6 Concentration of fruit juices by membrane technology

Nanofiltration

Nanofiltration is the most suitable separation method among the pressure-driven membrane processes for complex solutions, since by appropriate selection of the membrane, it can be successfully applied for the separation of compounds with similar molecular weight (Conidi et al., 2012).

The advantage, reported by the authors, of nanofiltration to reverse osmosis is in the higher permeate fluxes achieved in the filtration of complex solutions, and the better retention of compounds with similar molecular weight in the range of 150-1000 Da, such as sugars, natural organic compounds and ions, when compared to ultrafiltration. The efficiency of filtration in terms of the permeate flux is determined by the steric (sieving) and charge (Donnan) effects (Echavarriá et al., 2011).

Up to 84% rejection of phenolic compounds and 99% of flavonoids was achieved in the nanofiltration of the aqueous propolis extracts, and 53% of the phenolic compounds and 90% of the flavonoids in ethanolic solution when a membrane composed of polyamide and polysulphone with a 98% of MgSO$_4$ was used at pressure of 50 bar and temperature of 20 °C (Mello et al., 2010).

Conidi et al. (2012) used the permeate flux, rejection and fouling indices to describe the performance of four different spiral wound nanofiltration membranes with a NWCO values of 180, 300, 400 and 1000 Da, made of polyamide, polypiperazine amide and polyethersulphone in the process of recovery of phenolic compounds from orange press liquor. The process parameters were 20 bar and 20 °C in the case of the membrane with 180 and 300 Da, and 6 bar and 20 °C for the membranes with 400 and 1000 Da. The results indicated that a polyethersulfone membrane was susceptible to fouling and that the normal cleaning procedure was insufficient for the recovery of the membrane. Under these conditions, high anthocyanin (89.2-95.9%) and flavonol (70.0-95.4%) rejections were achieved for all of the NF membranes investigated. The rejection of sugars was more diverse, 22.8% rejection was measured in the case of the 1000 Da membrane, and 42.8% for the 400 Da membrane.
**Reverse osmosis**

In reverse osmosis, the mass transfer of the permeant is controlled by the solution-diffusion mechanism, in which the permeate dissolves in the membrane and then diffuses through the membrane. The reverse osmosis membranes are very hydrophilic (Wenten & Khoiruddin, 2016).

The concentration extent in reverse osmosis is limited, not only due to concentration polarisation and fouling phenomena, but also due to the increase in juice viscosity and osmotic pressure (Gurak et al., 2010; Echavarría et al., 2012).

The rate of concentration can also be affected by the amount of pectin compounds in the juice samples. In the study by Garcia-Castello et al. (2011), only a volumetric reduction factor of 1.2 could be achieved when a spiral wound membrane made of polyamide was used at temperature of 20 ± 1 °C, at pressure of 20, 35 and 50 bar and at the retentate flow rate of 300 L/h. The reason behind the low volumetric reduction factor achieved was due to the presence of pectin that maximised the fouling of the reverse osmosis membranes.

In order to reach higher efficiency in concentration described by the °Brix value of the end product, the operating pressure of the system must always be greater than the osmotic pressure of the feed.

The solute concentration at the membrane surface will merely determine the osmotic pressure value than the solute concentration in the bulk (Mulder, 1991). For instance, Gurak et al. (2010) reached the value of 28.5 °Brix in the concentration of grape juice when using a plate and frame module that contained a thin film composite with a 95% NaCl rejection at 50 °C and by applying pressure of 60 bar. On the other hand, Jesus et al. (2007) achieved 36 °Brix in orange juice concentration using a plate-and-frame module equipped with polysulphone/polyethylene composite layer membranes at 25°C and 60 bar pressure.
4 Extraction of anthocyanins from blackcurrant marc

In the past decade, the demand for novel extraction techniques has increased and this has led to a number of new extraction techniques, such as supercritical fluid extraction, ultrasound-assisted extraction, accelerated solvent extraction and microwave-assisted extraction.

These extraction techniques are intended to promote extraction efficiency by reducing the extraction time significantly, and to increase the extraction yield of a defined compound from the solid matrix into solution. A feature in common for all these techniques is operation at elevated temperature, which in turn speeds up the extraction process.

4.1 Conventional solvent extraction

Extraction is a diffusional process in which the component in question is separated from a solid or a liquid phase by a selective solvent. The separation can take place to separate one or more components contained in the solid phase. The components that are extracted and transferred to the liquid phase are called solutes, and the component that stays intact in the solid is called the inert.

The solvent is selective if it brings only the solute of interest into the liquid phase. In addition to selectivity, there are also other requirements for the solvents in solvent extraction systems: they must be economically affordable, non-corrosive, non-flammable and non-explosive and non-toxic.

The solvent extraction itself usually involves other processes, such as preparation of the solid material (e.g. grinding or drying), separation of the solvent-solute system and regeneration of the solvent. The extraction process is stopped once the system reaches equilibrium. There are three stages to reaching equilibrium in the extraction process (Ibarz & Barbosa-Cánovas, 2003): phase change of the solute from the solid into the liquid phase, diffusion of the solute in the solvent due to a concentration gradient and the transfer of the solute into the solution due to a concentration gradient.

The extraction process takes place until the solute is completely dissolved and the solution has a uniform concentration (Ibarz & Barbosa-Cánovas, 2003).

It is generally known that the yield of chemical extraction depends on the type of solvent (polarity), extraction time and temperature (Singh et al., 2014). In solvent extraction, the compounds of interest may degrade due to the high
temperatures applied and prolonged extraction time, and some solvents may also have some health-related risks (Condini et al., 2012).

The duration of extraction can be decreased by increasing the transfer rate, which can be achieved by decreasing the particle size, increasing the temperature and stirring the fluid (Ibarz & Barbosa-Cánovas, 2003). The main disadvantage of this technology is that the typically used solvents such as ethanol, methanol, acetone and ethyl acetate are not necessarily suitable for food applications (Amyrgialaki et al., 2014).

Despite these disadvantages, solvent extraction has found a niche in food industrial application. Extraction with water can be used in processing sugar beet to sugars and in extraction of roasted coffee beans to produce instant coffee and of tea leaves to produce instant tea. Besides, it is used in vegetable and fish oil separation from e.g. low-value fish or fish liver.

4.2 Microwave-assisted extraction

Azmir et al. (2013) reported that microwaves are characterized with a frequency range between 300 MHz to 300 GHz. In microwave-assisted extraction the extraction time is reduced, which is due to the difference in heating requirement compared with conventional heating. In conventional heating, the vessel is heated and a finite time is required until the heat is transferred to the solution. In the microwave-assisted technique, the solvent is heated directly, resulting in a minimum temperature gradient, and this accelerates the speed of heating.

Heating by microwaves is volumetric and selective (Galan et al., 2017). Volumetric heating is responsible for the rapid heating times, while selective heating results in a component change in the mass transfer process due to a heat gradient. The principle of heating using microwave energy is based on the direct effect of microwaves on molecules by ionic conduction and dipole rotation. Ionic conduction is the migration of ions when an electromagnetic field is applied. The resistance of the solution to this flow of ions results in friction and thus heats the solution. The dipole rotation means realignment of dipoles with the applied field. At 2.45 GHz, the dipoles align and randomise $4.9 \times 10^9$ times per second and this forced molecular movement results in heating.

The microwave-assisted extraction process involves three sequential steps (Alupului et al., 2012): solute separation from the sample matrix under increased temperature and pressure, diffusion of solvent across the sample matrix and the release of the solutes from the sample matrix to the solvent. The dissipation
factor (\(\tan \delta\)) determines the ability of a solvent to absorb the microwave energy and passes it in the form of heat to the desired molecules. The dissipation factor can be determined from the ratio of the efficiency of converting microwave energy into heat (i.e. dielectric loss) to the polarisability of a molecule in the electric field (dielectric constant) and can be calculated based on the following equation:

\[
\tan \delta = \frac{\varepsilon''}{\varepsilon'}
\]

where \(\varepsilon''\) is the dielectric loss and \(\varepsilon'\) is the dielectric constant (Datta & Ananrheswaran, 2001).

The dielectric properties of materials are a very important factor that determines the penetration of energy. In the extraction process the dominant material is the solvent, which mostly consists of water, so the properties of water are the determining factor. The dielectric properties of the material are also dependent on structural properties such as the density, composition and temperature (Datta & Ananrheswaran, 2001). Above all, the behaviour of a material in a microwave treatment will depend on the presence of mobile ions and permanent dipole moments (Datta & Ananrheswaran, 2001).

In microwave-assisted extraction, a suitable solvent should be chosen based on the solubility of the target compound, the solvent’s penetration ability and the composition of the matrix (Chan et al., 2011). In many cases the solvent is an aqueous solution of a certain organic solvent, because of the easy penetration of the energy due to the water content. In other cases, ethanol, methanol or acetone can be used (Chan et al., 2011). The most important issue in selecting the solvent in this case, as in the case of the solvent extraction, is that it must be non-toxic.

Microwave-assisted extraction is gaining importance in the field of extraction processing due to the fact that it can be easily applied at both small and larger (up to industrial) scale (Cravotto et al., 2008). As examples, solvent-free microwave extraction of essential oils from aromatic herbs and separation of volatile and nonvolatile organic compounds in boldo leaves have been proposed for possible industrialisation (Barba et al., 2016).
5 Materials and methods

5.1 Materials

In the following sections, the different materials used in Papers I-IV are described.

5.1.1 Blackcurrant juice

The blackcurrant juice used in the experiments and described in Paper II was produced by Fitomark 94 Ltd, Hungary. The juices were treated with enzymes to make the pressing easier and to increase the yield. The enzymes used for this are described in section 5.1.2. The berries were pressed using a Pera PN BUCHER compressor. The juice was then pasteurised and clarified conventionally by centrifugation.

The blackcurrants used in Papers I and III were of the Öjebyn variety and were cultivated in the Alavieska area of Finland. Blackcurrants produced in 2004 were used for the ultrafiltration study (Paper I) and blackcurrants produced in 2006 for the reverse osmosis experiments (Paper III). All the berries were pressed in the same small-scale berry processing company (Marjarannikko Ltd., Raah, Northern Finland) using identical methods and equipment (Papers I and III).

The berries were first crushed in a centrifugal fruit mill (Enotecina pillan s.n.c., Italy), in order to accelerate and improve the enzyme treatment. The berries were kept at temperature of 45 °C for 3 hours in a tank fitted with a temperature control shell. Berry pressing was done using a hydraulic bag press (Drink Consult Finland Inc., Finland) at 380 bar pressure. After pressing, the juice was pasteurised at 85 °C with a plate heat exchanger (Karl Bockmeyer Kellereitechnik GmbH, Germany), packaged and cooled in 10 L polyethylene canisters. The juice was then stored in the canisters at -16 °C until further processing.

The anthocyanin and flavonol content of the blackcurrant juice used in Paper III is shown in Table 4.
Table 4. Total anthocyanin and flavonol content in the blackcurrant juice samples used in Paper III. Total anthocyanins are the sum of Dp-3-glu, Dp-3-rut, Cya-3-glu and Cya-3-rut and total flavonols are the sum of myricetin and quercetin.

<table>
<thead>
<tr>
<th>Juice sample</th>
<th>Total anthocyanin content (mg/L)</th>
<th>Total flavonol content (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OJ1</td>
<td>835.8</td>
<td>42.9</td>
</tr>
<tr>
<td>OJ2</td>
<td>884.4</td>
<td>53.1</td>
</tr>
<tr>
<td>OJ3</td>
<td>969.0</td>
<td>64.7</td>
</tr>
<tr>
<td>UFJ</td>
<td>333.4</td>
<td>40.6</td>
</tr>
</tbody>
</table>

OJ1, OJ2 and OJ3 = three different batches of the original pressed juice, UFJ = the blackcurrant juice after ultrafiltration.

5.1.2 Enzymes used

Pectinolytic enzymes are widely used in the fruit juice industry, mainly for increasing the yield during juice extraction. In addition, they have an impact on the colour release during this process step.

In this thesis three different enzyme preparations were used, with the main aim to make the filtration process easier in the ultrafiltration and reverse osmosis steps. The main characteristics of the enzymes are summarised in Table 5. Before ultrafiltration, the blackcurrant juice samples were pre-treated with a Panzym Super E liquid (E. Begerow GmbH & Co., Germany) pectinase enzyme product (Paper I). Trenolin enzyme (Erbslöh Geisenheim AG, Germany), Panzym Super E and Rohapect berry (AB Enzymes, Germany) were used in the reverse osmosis experiments (Paper II and Paper III).

Panzym Super E and Rohapect berry differ from each other in their main and side enzyme activities. Panzym Super E is a highly concentrated special pectinase preparation for fruit juice treatment, with an enzyme activity of 3000 PECTU/mL. It is obtained from selected strains of *Aspergillus niger* with very broad activity spectra comprising the pectinases, hemicellulases, cellulases and arabanases (Begerow, 2006).

Rohapect berry is a novel enzyme mixture, and is specially developed for the treatment of berry juices. It is not available for commercial purposes, since the information is based on a personal communication with the AB Enzymes company. It has a main pectinase enzyme activity of 150 PTF/mg, with arabanase side activity.
Trenolin is an easy-to-dose pectolytic enzyme. Usage effects the early release of colouring matter and thus considerably shortens the remaining stages of the mash necessary for liberation of these substances. Undesired oxidation with negative effects is thereby prevented. Trenolin is an enzyme product purified in a special process and is therefore free from disturbing depsidase and oxidase side activities (Erblslöh, 2017).

Table 5. Properties of enzymes used in this thesis.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Enzyme activity</th>
<th>Main activity</th>
<th>Side activity</th>
<th>Time (h)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panzym Super E</td>
<td>3000 PECTU/mL</td>
<td>Pectinase</td>
<td>Hemicellulase</td>
<td>2-6 (Paper I)</td>
<td>55 (Paper I)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cellulase</td>
<td>12 (Paper II)</td>
<td>25 (Paper II)</td>
</tr>
<tr>
<td>Trenolin</td>
<td>min. 7.2 ASV-U/mL</td>
<td>Pectinase</td>
<td>N.A.</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>Rohapect berry</td>
<td>150 PTF/mg</td>
<td>Pectinase</td>
<td>Arabanase</td>
<td>96</td>
<td>6</td>
</tr>
</tbody>
</table>

5.1.3 Blackcurrant pressing residues

After squeezing the blackcurrants, the pressing residue (marc) was stored frozen at -22 °C in polyethylene bags until extraction. The average total solids content of the marc on a wet weight basis was 23.5% (w/w%). The marc contained the skins, seeds and pulp of the blackcurrants.

5.1.4 Ultrafiltration and reverse osmosis membranes

In the ultrafiltration experiments in Paper I, a Biomax 100 kDa hydrophilic polymeric membrane were used. For clarification of the blackcurrant juices, the ultrafiltration equipment contained a Pellicon 2 module equipped with a Biomax membrane with an active membrane area of 0.1 m², with a nominal weight cut-off value (NWCO) of 100 kDa.

The membrane was made of polyethersulfone and permitted the utilisation of transmembrane pressure up to 7 bar and an operating temperature between 4 and 50 °C. The Pellicon 2 filters and holders were reported to be suitable for polysaccharide solution processing in the manufacturer fact sheet more efficiently due to the tangential filtration flow performance.
The reverse osmosis experiments were carried out with equipment that contained a B1 tubular module. The module was equipped with AFC 80 in Paper II or AFC 99 in Paper III, both made from polyamide film, with a maximum transmembrane pressure of 60 and 64 bar and an maximum allowed operating temperature of 70 and 80 °C, respectively.

This membrane was chosen because of its suitability for feedstock with a high suspended solids content, which will allow that no or minimum pre-treatments are required to achieve high filtration efficiency. The benefit of the membranes relies on the module configuration, since the tangential flow of the material in such module allows a better efficiency to treat materials with a high dissolved solid or suspended solid content. Tubular configurations are usually less susceptible to fouling than other configurations. They are also an absolute priority for more intensive chemical and mechanical cleaning methods when compared to other configurations as reported by Mulder (1991).

5.2 Methods

This section describes the enzyme treatment applied to the blackcurrant juices. The ultrafiltration and reverse osmosis equipment and procedures, as well as the extraction processes, are also described.

5.2.1 Enzyme treatment of blackcurrant juices

In the ultrafiltration process, Panzym Super E was inoculated into the juice batches at a concentration of 150, 200 and 250 mL/100 L juice and the mixture was kept for different times at 55 °C until analysis confirmed that pectin was not present in the blackcurrant juice.

In all reverse osmosis studies, small amounts of enzyme (8 mL/20 L juice) were used, but the treatment times and temperatures were varied. In Paper II, the treatment time was 12 hours for Panzym Super E at 25 °C, while for Trenolin it was 24 hours at 25 °C and 96 hours at 6 °C. In Paper III, Panzym Super E and Rohapect berry were dosed in a concentration of 8 mL/20 L juice, and the juices were kept at 45 °C for 2 hours.

Optimisation of the enzyme dosage was excluded in this research, and all the juices were heated up at the end of the enzyme treatment to 60 °C to inactivate the enzyme.
5.2.2 Ultrafiltration

Ultrafiltration experiments were carried out by a tangential flow filtration system on a laboratory-scale system (ProScale™ system, Millipore, France) that is suitable for micro-, ultra- and nanofiltration, as well as concentration by reverse osmosis. The equipment has an integral tubular heat exchanger to ensure constant operating temperature. This system is ideal for processing liquids in volumes up to 10 L.

In the ultrafiltration process, the blackcurrant juice was pumped by a high pressure diaphragm pump (0-40 bar, 0-600 L/h, 1.5 kW) into a Pellicon 2 Mini Cassette membrane module with a Biomax 100 kDa membrane. The experiments were carried out in both total recycle mode and batch concentration mode. Total recycle mode was used to determine the best operating parameters and during these experiments both the permeate and retentate streams were returned to the feed vessel to maintain constant volume conditions (Figure 6). In the batch concentration, the permeate was continuously removed and collected.

![Diagram of total recycle and batch concentration operating modes](image)

Fig. 6. Schematic diagram of the total recycle and batch concentration operating modes (Paper I © 2012 Springer).

In subsequent experiments the batch concentration mode was used and the transmembrane pressure was set to $\Delta P_{\text{TM}} = 2$ bar by a recirculation flow rate of
Q = 220 L/h. The amount of feed in each case was 5 L. In batch concentration mode, the permeate was continuously removed and collected, while the retentate was recycled to the feed vessel until a certain volumetric reduction factor was reached.

Cleaning of the membrane was carried out after every test run as follows: first, the membrane was rinsed with ultrapure water (Milli-Q, Millipore, France) for 30 min, followed by cleaning with an enzymatic detergent (Tergazym, Alconox Inc., USA) solution in 0.2% (w/w %) concentration and, finally, with NaOCl solution (Kloriitti-Forte, Farmos Teknokemia, Finland) in a concentration of 250 ppm. These solutions were circulated for 60 min, both at 45 °C and at a recirculation flow rate of 110 L/h. A final rinse with ultrapure water was then carried out for 15 min. The water flux was measured at 24 °C before and after each experiment, and after cleaning.

5.2.3 Reverse osmosis

In Paper II, an RO tubular B1 module from Paterson Candy International which comprises 18 perforated stainless steel tubes, was used. Each tube was lined with a 1.2 m long membrane element 12.5 mm in diameter (total area of 0.9 m²). The tubes were connected in series. The module was equipped with an AFC 80 polyamide tubular membrane.

This compact tubular method was developed to facilitate concentration of highly viscous fluids. The temperature of the feed was controlled by a heat exchanger and set to 25 °C. After the juice was fed through the membrane module, the concentrate was recirculated back to the storage tank (Figure 7). The transmembrane pressure was fixed at 60 bar, and 60 L of juice were concentrated in each batch with a recirculation flow rate of 900 L/h, resulting in a flow velocity of 2.04 m/s. The flow velocity was calculated using the cross-sectional area of the membrane and the recirculation flow rate. Therefore, the concentration took place in the turbulent flow regime where the velocity is only smaller in the boundary layer near the membrane wall (Mulder, 1991).

Cleaning of the membranes was carried out after every test run as follows: the membrane was first rinsed with tap water at a recirculation rate of 25 L/h and a trans-membrane pressure of 60 bar for 30 min. This was followed by circulating 0.1% (w/w%) ethylenediaminetetraacetic (EDTA) - sodium-dodecyl-sulphate (SDS) - sodium-hydroxide (NaOH) solution in the same conditions for 30 minutes and rinsing with tap water. Finally, a 0.5% (w/w%) citric acid solution
was circulated for 30 min, followed by rinsing with tap water. The pure water flux was measured before and after each cleaning procedure, and used later in calculation of the total resistance from the resistance-in-series model.

In Paper III, reverse osmosis was carried out using pilot-scale Paterson Candy International equipment (Hyxo Ltd., Finland, Figure 7). The B1 module of the equipment comprises 18 perforated stainless steel tubes. Each tube is lined with a 1.2 m long membrane element 12.5 mm in diameter and the tubes are connected in series. The membrane was an AFC-99 thin film composite membrane with an effective membrane area of 0.9 m².

The membrane used for the experiments was new, and therefore, it was conditioned by circulating distilled water for 2 hours at a temperature of 25 °C and a pressure of 20 bar. The pure water flux of the membrane was calculated by adjusting different transmembrane pressures between 15 and 55 bar at 20 °C. The pure water fluxes were measured in duplicate after the conditioning to evaluate the stability of the fluxes.

The concentration of the blackcurrant juices were carried out at transmembrane pressure of 45 bar, at temperature of 30 °C, with a recirculation flow rate of 600 L/h for approximately one hour. Based on the calculations, the flow velocity value was 1.35 m/s in this concentration trials, which will result in a turbulent flow in the membrane also in this case similarly when the concentration test was done in the equipment holding the AFC 80 membranes.

Following the concentration, the membrane was rinsed with distilled water for 20 minutes so that the permeate and retentate were directed to the drain, and the tank of the equipment was filled again with distilled water when it was approximately half-full. After the rinsing process, the pure water flux was recorded again. No cleaning with chemicals was needed. All the experiments and the analyses were performed in duplicate.

Both the AFC 80 and AFC 99 membranes are made of polyamide material, but their benefit merely relies on the module configuration, since the tangential flow of the material in such module allows a better efficiency to treat materials with a high dissolved solid or a suspended solid content. Tubular configurations are also less susceptible to fouling usually other configurations. In addition, they are an absolute priority for more intensive chemical and mechanical cleaning methods when compared to other configurations as reported by Mulder (1991).

Although compaction problems in the reverse osmosis process may arise due to the utilization of the relatively high pressures, which will result in the deformation of the membranes and therefore the flux values will not return to
their original values, reference to this phenomenon was not observed during the concentration of the blackcurrant juices when fluxes before and after the experiments were compared.

Fig. 7. Schematic diagram of the reverse osmosis equipment.

5.2.4 Conventional extraction

An Armfield pilot plant solvent extractor (Hampshire, UK) was used for the conventional extraction (CEx) experiments in Paper IV (Figure 8). The extraction was carried out at a constant temperature of 80 °C.

The solvents used were aqueous HCl at pH 2, citric acid solution at pH 2 and a solution containing 50 ppm of SO₂ and 1% (w/w%) of citric acid. A 100 g portion of blackcurrant press residue was used as an experimental batch. The marc to solvent ratio was 1:40 and the flow rate of solvent circulation was adjusted to 0.33 L/min.

The temperature for the conventional extraction was chosen based on the preliminary experiments in the microwave-assisted extraction. In these experiments, the temperature of the extracts, measured with an infrared temperature probe at the surface of the solvent, was approximately 80 °C at the end of the extraction.
5.2.5 Microwave-assisted extraction

The microwave-assisted extraction test in Paper IV was carried out using a single-mode cavity resonator at a magnetron frequency of 2.45 GHz (Figure 9). The power output of the magnetron can be adjusted between 100 to 700 W by varying the anode voltage.

In all experiments, 28 g of marc was used. Response surface methodology was used to determine the optimum conditions for the extraction to achieve the highest anthocyanin content in the extract.

During the extraction, the microwave power was varied between 140 and 700 W, the pH of the solvent between pH 2 and pH 7, the marc to solvent ratio between 1:10 and 1:20 and the extraction time between 10 to 30 min.

Polytetrafluoroethylene (PTFE) vessels were used to minimise energy loss of microwave irradiation. The vessels were covered to prevent evaporation during the irradiation without a pressure increase.
Fig. 9. Schematic diagram of the microwave-assisted extraction equipment: 1) Toroidal core transformer, 2) high voltage power transformer, 3) magnetron, 4) rectangular waveguide, 5) waveguide-resonator spreader, 6) cavity resonator holding the sample, 7) waveguide tuning screws, 8) perforated-plate top, 9) directional coupler, 10) diode power sensor (NRVZ, Rohde & Schwarz), 11) power meter (NRVD, Rohde & Schwarz) and 12) hydraulic elevator.

5.2.6 Response surface methodology

Response surface methodology (RSM) consists of mathematical and statistical methods for setting up an empirical model to describe the process. The usual main aim of RSM is to optimise a response, which can be any kind of output variable. This output variable is influenced by several input (independent) variables.

Optimisation is usually carried out by altering one variable at a time, while the others are kept constant, and observing the effect on the response. However, this method is time-consuming and also neglects the interactive effects between the input variables and their role in the optimisation. In addition, the number of experiments needed is very high. The objective of RSM is to simultaneously optimise the levels of variables to achieve the best system performance (Bezzerra et al., 2008).

An important part of RSM is the design of experiments. To carry out this task, first the independent variables are determined with their minimum and maximum values and the response is defined. This is followed by building up the experimental design and carrying out the experiments. Once the values for the responses are known, the mathematical-statistical technique is applied through fitting a polynomial equation. After this step, the evaluation of the model begins,
in which the fitness of the model is analysed and ANOVA is performed. If no lack of fit is found in the analyses and the model is statistically good, optimisation can be carried out. However, this may sometimes require additional experiments to be conducted in the sphere of the optimal region. Finally, when the optimum point is found for each independent variable studied, verification of the model is performed by carrying out parallel experiments at the optimum point.

The response surface methodology was used in Paper IV to determine the optimum conditions for anthocyanin extraction from blackcurrant marc. The effects of the following factors were investigated: microwave power (MWP, $X_1$), the marc to solvent ratio (S:L, $X_2$), the pH of the solvent (pH, $X_3$) and the extraction time (t, $X_4$). As a response function, the monomeric anthocyanin pigment (MAP) content of the extracts was measured. The monomeric anthocyanin pigment yield was calculated and expressed in mg/g as cyanidin-3-glucoside equivalents.

The optimisation procedure was performed after refinement of the model with the aim of defining processing conditions for maximum recovery of anthocyanins from the blackcurrant marc.

The complete design consisted of 29 runs, including five replicates in the center points (exp. No 25-29) to evaluate the model validity. The experiments were carried out in a randomised order, to avoid unexplained variability in the response due to systematic error. The design and the response values obtained are listed in Table 6.

A central composite face-centered (CCF) design was applied in Paper IV and a polynomial equation was fitted by multiple linear regression, since this method is suitable when there is only one response at time.

The multiple linear regression model is described as:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k + \varepsilon \quad (15)$$

where $Y$ is the dependent variable, $\beta_j = 0, 1, \ldots, k$, are the regression coefficients of the model and $x_i, i=1,2,\ldots,k$ are the independent variables in the model.
Table 6. Statistical experimental design used for extraction of anthocyanins from blackcurrant marc. MWP = microwave power, S:L = marc to solvent ratio (S:L, $X_2$), pH refers to pH of the solvent and t = extraction time (Paper IV © 2013 Springer).

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>MWP (W)</th>
<th>S:L</th>
<th>pH</th>
<th>t (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>140</td>
<td>0.05</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>N2</td>
<td>700</td>
<td>0.05</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>N3</td>
<td>140</td>
<td>0.1</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>N4</td>
<td>700</td>
<td>0.1</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>N5</td>
<td>140</td>
<td>0.05</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>N6</td>
<td>700</td>
<td>0.05</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>N7</td>
<td>140</td>
<td>0.1</td>
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</tr>
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<td>N8</td>
<td>700</td>
<td>0.1</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>N9</td>
<td>140</td>
<td>0.05</td>
<td>2</td>
<td>30</td>
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<td>4.5</td>
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</tr>
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<tr>
<td>N29</td>
<td>420</td>
<td>0.075</td>
<td>4.5</td>
<td>20</td>
</tr>
</tbody>
</table>
5.3 Analytical methods

5.3.1 Total soluble solids

Total soluble solids (TSS) were measured and expressed as °Brix using an H-50 refractometer (Atago, Japan) with a scale range of 0-50 °Brix in Paper II and an Atago PR-100 Palette (Atago Co Ltd., Japan) digital refractometer (0-32 Brix%) in Paper I. Measurements were made at ambient temperature and, prior to each set of measurements, the instrument was calibrated to 0 °Brix using distilled water.

5.3.2 Viscosity

Viscosity of the blackcurrant juice samples was measured in Paper II using an SV10 vibrational viscometer (A&D Instruments Ltd.).

5.3.3 Density

Density of the blackcurrant juice samples was determined in Paper II using an oscillometric Densito PX30 density meter (Mettler Toledo GmbH).

5.3.4 Analysis of anthocyanins and flavonols by HPLC

In Paper I, anthocyanins and flavonols were analysed by high performance liquid chromatography (HPLC) using an Agilent Technology apparatus (Agilent 1100 Series HPLC) connected to a diode array detector (DAD) and a mass selective detector (MSD). A Hypersil ODS column (2.1×200 mm, 5 μm; Agilent Technology) and a gradient run (0.5 mL/min) with acetonitrile (A) and 10% formic acid (B) were used for separation of the compounds in three replicates of each sample. The solvent programme for anthocyanins was as follows: 95% B from 0 to 2 min, followed by linear gradients of 95-85% B from 2 to 20 min, and 85-80% B from 20 to 30 min.

The solvent programme used for flavonols was: 90% B from 0 to 2 min, followed by linear gradients of 90-70% B from 2 to 10 min, and 70-50% B from 20 to 30 min.

For anthocyanin analysis, the samples were diluted with ultrapure water by a factor of two, filtered through an 0.45 μm syringe filter and analysed directly by HPLC/DAD/MSD (API-ES with positive mode). Peaks were identified using 3-
O-glucosides of cyanidin, delphinidin, peonidin and malvidin (Polyphenols, Norway) as standards, with MSD spectra and retention times, and comparing the data obtained against literature values. The concentrations of anthocyanins were calculated (from DAD data obtained at 520 nm) using cyanidin-3-glucoside (Polyphenols, Norway) as the external standard.

The flavonols studied, quercetin and myricetin, were analysed as aglycons after acidic hydrolysis by adding HCl (1 M) to the sample (1:1 v/v%) and incubating the sample for 2 h at 70 °C. When necessary, the samples were diluted and filtered through a 0.45 μm syringe filter for HPLC. The concentrations were calculated (from DAD data obtained at 365 nm) using external standards of quercetin and myricetin (Sigma-Aldrich Chemie Ltd., Switzerland).

In Paper III, anthocyanins and flavonols (two replicates for each treatment) were identified and analysed by HPLC (Agilent 1100 Series HPLC, Agilent Technology) connected to a diode array detector and a mass selective detector using a Hypersil ODS column (2.1 mm x 200 mm, 5 μ, Agilent Technology) for the ultrafiltered and enzyme-treated juices and a Hyperclone ODS column (2.0 mm x 200 mm, 5 μ, Phenomenex) for the centrifuged juice. Solvent A was acetonitrile and solvent B was 10% formic acid.

The gradient used for anthocyanin analysis was 95% B from 0 to 2 min, followed by linear gradients of 95-85% B from 2 to 20 min, and 85-80% B from 20 to 30 min, with a flow rate 0.5 mL/min. Peaks were identified using 3-O-glucosides of cyanidin, delphinidin, peonidin, and malvidin (Polyphenols, Norway) as standards, with MSD spectra and retention times, and comparing the data obtained against the literature. The concentrations of anthocyanins were calculated (from DAD data obtained at 520 nm) using cyanidin-3-glucoside (obtained from Polyphenols, Norway) as an external standard.

The solvent programme used for flavonols was: 90% B from 0 to 2 min, followed by linear gradients of 90-70% B from 2 to 10 min, and 70-50% B from 20 to 30 min.

The flavonols studied, quercetin and myricetin, were analysed as aglycons after acidic hydrolysis by adding 1 M HCl to the sample (1:1, v/v%) and incubating the sample for 2 h at 70 °C. Samples were diluted when necessary and filtered through a syringe filter with pore size 0.45 μm. The concentrations were calculated (from DAD data obtained at 365 nm) using external standards of quercetin and myricetin (obtained from Sigma Aldrich, Finland).

In Paper IV, the anthocyanin composition of the extracts was determined using HPLC as follows. A mixture of 5 mL of extracted sample and 5 mL 2%
acetic acid was subjected to 15 minutes vortexing and 1 minutes homogenisation in an ultrasonic bath. The samples was prefiltered through a 0.45 mm polyamide filter (Macherey-Nagel) into a vial prior to injection into the HPLC. The HPLC method used by Goiffon et al. (1999) was followed, with a modification to separate and quantitatively determine anthocyanins on an Sphinx RP 100 C18 3μm 150× 4.6 column (Macherey-Nagel, Germany), using gradient elution of (A) 10% formic acid in water and (B) 10:45:45 formic acid-water-acetonitrile as the mobile phase (flow rate 0.7 mL/min). The elution started with 100% A, changed to 100% B after 25 minutes and changed to 100% A after 5 minutes. For analysis of anthocyanins, a Waters (Alliance) HPLC system consisting of a 2695 Separation Module and a 2996 photodiode-array detector (PDA) was used. Operation and data processing were performed using Waters Empower software.

For quantification, the HPLC chromatogram was displayed and integrated at 525 nm. Diluted solutions of cyanidin-3-rutinoside (from Sarsynthese Marignac, France) were used as external standard to quantify the peaks and in the calibration and recovery test. The total anthocyanin content was measured by a spectrophotometric method.

### 5.3.5 Analysis of monomeric anthocyanin pigments by the pH difference method

In Paper IV, monomeric anthocyanin pigments were determined using a pH difference protocol. This method relies on structural transformation of the anthocyanin chromophore as a function of pH, which can be measured by spectroscopy.

Samples from all 54 experimental runs were collected, and 100 μL of extract were diluted to 1000 μL with distilled water. Two dilutions of the samples were made, one with a potassium chloride buffer at pH 1.0 and the other with a sodium acetate buffer at pH 4.5. The pH of the buffers was adjusted with HCl solution (Spektrom-3D, Hungary). The dilution factor was determined in accordance with the linear range of spectrophotometers. The dilutions were left for 30 minutes to equilibrate at 20 °C, and the absorbance was measured at 520 nm and 700 nm with an UV/VIS spectrophotometer (WPA Lightwave S2000, UK) in quartz cuvettes against distilled water. The difference in absorbance of the diluted samples was calculated as:

\[
A = (A_{\text{vismax}, 520} - A_{700})pH_{1.0} - (A_{\text{vismax}, 700} - A_{700})pH_{4.5}
\]  

(16)
The monomeric anthocyanin pigment (MAP) content, expressed as cyanidin-3-glucoside equivalents, was calculated as:

\[
MAP = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1} \quad \text{(mg/L)}
\]  

(17)

where \( MW \) is the molecular weight (= 449.2), \( DF \) is the dilution factor and \( \varepsilon \) is the molar absorptivity (= 26900).

The anthocyanin yield \((c)\), expressed as mg/g wet weight marc, was calculated as:

\[
c = \frac{c_{\text{anthocyanin}} \times V_{\text{solvent}}}{m_{\text{marc}}} \quad \text{(mg/g)}
\]  

(18)

### 5.3.6 Statistical analysis

Statistical analysis was carried out using the Statistica Programme. The data were analysed using analysis of variance (ANOVA), with differences considered to be statistically significant at \( p \leq 0.05 \).
6 Results and discussion

6.1 Effect of pre-treatment on ultrafiltration of blackcurrant juice

This chapter presents the results of clarification by the ultrafiltration process. First, the effects of pre-treatment in helping to increase the permeate flux in the ultrafiltration process and the effects of the enzymatic depectinisation are presented. Preservation of anthocyanins and flavonols in the clarified juice is then described.

6.1.1 Effect on permeate flux

In Paper II, one of the aims was to investigate the effect of enzyme treatment on permeate flux in the ultrafiltration process. Pectolytic enzymes hydrolyse the methyl ester groups of the pectin and reduce the viscosity of the juice, which in turn raises the permeate flux during filtration. Benefits of depectinisation have also been observed in ultrafiltration of apple juice (Alvarez et al., 1998) and other juices (Sahin & Bayindirli, 1993; Rai et al., 2007a, 2007b; Maktouf et al., 2014).

The results from the filtration are illustrated in Figure 10 for experiments carried out at a recirculation flow rate of 200 L/h and a pressure of 2 bar until a volumetric reduction ratio of 8.3 (or 5.6 at 45 °C) was reached. The data on the permeate flux is presented in the function of VRF values.

Temperature of particular interest was 25 °C, due to the heat sensitivity of anthocyanins. Cacace & Mazza (2003) report that a sharp decrease in the anthocyanin content of juices occurs at high temperatures. The authors reported that the critical temperature for anthocyanin extraction is about 35 °C and a sharp decrease is observed at temperatures beyond 45 °C. Casani & Bagger-Jørgensen (2000) reported that at as low temperature as 4 °C, the precipitation of polyphenols and proteins can be avoided, and the fouling of the membrane can be prevented.

The reason for filtration at higher temperature in this work, i.e. at 45 °C, was to determine the increase in permeate flux as the temperature increases. At higher temperatures the viscosity of the juice is lowered, which results in better filtration efficiency. However, at this temperature a continuous flux decline was observed, steady-state conditions failed to develop and the membrane was eventually fouled.
The normalized water flux (NWF) values were calculated before and after the ultrafiltration, as well as after the cleaning procedure. In the case of ultrafiltration of the blackcurrant juice treated with 200 mL/100 L juice Panzym Super E at 45 °C, the initial NWF value was 7.59, and after the ultrafiltration 0.45. After the cleaning procedure, the NWF value was recovered to 7.08.

When the NWP data from the ultrafiltration of different samples is compared, it can be observed that the most significant drop in NWP values happened in the filtration at 45 °C. In the case of filtration at 25 °C, the initial NWF value was 6.45, after the ultrafiltration process 0.8, and after the cleaning procedure 6.1.

The NWF value in the case of the sample treated with 150 mL/100 L juice Panzym Super E, the initial value dropped from 7.24 to 1.053 after the filtration, and was recovered to the value of 7.16 after the membrane cleaning procedure. From the point of view of efficiency of the membrane cleaning process, the membrane was recovered with the same efficiency. After the test at higher temperature, the NWF value was restored to 93% of the initial NWF value, and at lower temperature to 94%, respectively.

![Fig. 10. Permeate flux at different enzyme concentrations and operating temperatures in ultrafiltration using Biomax 100 kDa membrane (Q = 220 L/h and ∆P_{mt} = 2 bar) (modified from Paper I © 2012 Springer).](image)
The aim of the depectinization process was to improve the permeate flux. The Panzym Super E enzyme was dosed at 150, 200 and 250 mL/100 L juice and a moving average of the permeate fluxes was calculated. The highest permeate flux was achieved at 200 mL/100 L, with an average value of 42 L/m²/h, and it was found that increasing the concentration of the enzyme to 250 mL/100 L juice did not result in any further increase in the permeate flux and led to an average value of 41 L/m²/h. With low enzyme levels, the average permeate flux was 36 L/m²/h. Based on these results, the juices obtained with the Panzym Super E dose of 200 mL/100 L juice were analysed for anthocyanin and flavonol content.

Even though, the economic prospect of the processing will be highly influenced by the performance efficiency of the ultrafiltration in terms of a permeate flux, the avoidance of fouling of the membrane and thus the shut-offs of the equipment for possible membrane replacement, the use of enzymes also should be optimized. It is crucial to find the minimum level of pectinase to be used for efficient filtration, and through that to reduce the costs arising from the depectinization as much as possible.

6.1.2 Effect of ultrafiltration on the anthocyanin and flavonol content of blackcurrant juice

The effect of ultrafiltration was evaluated as a clarification process. The changes in anthocyanin content after ultrafiltration are depicted in Figure 11 and the changes in flavonol content in Figure 12.
The anthocyanin content increased slightly during enzyme treatment. Buchert et al. (2005) concluded that the depectinisation process increases the yield of the juice, but also the transfer of valuable compounds into the juice.

According to Cassano et al. (2011), the ultrafiltration process has an effect on the anthocyanin content of pomegranate juice. Those authors found that the anthocyanin content decreased by 11.7%, which they attributed mainly to the removal of del-3-glu, cya-3-glu and pel-3-glu. In contrast, the results in this thesis showed that the retention of the major anthocyanins in blackcurrant juice were equally in the ultrafiltration process, and showed a much greater extend with an approximate value of 50%.

The comparison with the work of Cassano et al. (2001) was done to support and emphasize that ultrafiltration does effect on the anthocyanin content of the clarified juice. The significant difference in the extent of the anthocyanin retention can be supported by the complexity of membrane technology performance in which the overall performance will depend on many factors. In the two studies compared, the applied membranes in terms of membrane material and cut-off value were diverse. Cassano et al. (2001) used modified poly(etheretherketone) hollow fiber membrane characterized by 10% dextran 68 800 MW rejection, while in the ultrafiltration of the blackcurrant juice in
present study, polyethersulfone membrane with a cut-off value of 100 kDa was used. In addition, the equipment set up and design, as well as the operational parameters were diverse in the two studies which may play a role in the difference in results. Cassano et al. (2001) worked at a transmembrane pressure of 0.96 bar, at a temperature of 25 °C and a recirculation flow rate of 1166 mL/min. In contrast, in the study of blackcurrant juice ultrafiltration, the transmembrane pressure was 2 bar, the temperature of filtration either 25 or 45 °C, and the recirculation flow rate 220 L/h. The two juices under study were different. This work describes the ultrafiltration results of the blackcurrant juice, while Cassano et al. (2001) reports the results of the pomegranate juice quality after ultrafiltration. The pre-treatment of the two types of juices was different. In this study, enzymatic depectinization was used as pre-treatment, while in the case of pomegranate juice, only a pre-filtration with stainless steel filters was performed.

When investigating the effect of processing on the flavonol content, it was observed that the enzyme pre-treatment resulted in a significant increase in these compounds. The opposite effect was seen in the ultrafiltration process, in which the total flavonol content decreased by about 52%, the process being more selective for the myricetin than for quercetin compounds (Figure 12).

Fig. 12. Content of myricetin and quercetin in the original juice before ultrafiltration, and in the UF feed and UF permeate using Biomax 100 kDa membrane at an operational parameters of 2 bar and 200 L/h recirculation flow rate.
The possible explanation for this observation is the formation of protein-phenolic complexes, or the formation of complexes of phenolic compounds with the enzymes applied for the pre-treatment of the juice. Heavy fouling of the membrane was not observed after the temperature of processing was reduced from 45 °C to 25 °C, but it is possible that there is a gel layer buildup of the juice compounds on the membrane surface that may also play a role in the retention of the valuable compounds of the blackcurrant juices. Finally, it is also possible that the charge effect will play a role in the retention of valuable substances during the ultrafiltration process.

6.2 Concentration of blackcurrant juice by reverse osmosis

This section presents the data obtained on concentration of blackcurrant juice in the reverse osmosis process. The effects of enzyme pre-treatment and centrifugation were analysed both for the filtration efficiency and for the changes in the anthocyanin and flavonol content of the juice concentrates. The reverse osmosis process was modelled using the resistance-in-series and osmotic models.

6.2.1 Permeate flux during concentration of different feedstock samples

The permeate fluxes in the reverse osmosis process were calculated in Paper II and are depicted in Figure 13. There were marked differences in the permeate flux of the different pre-treatments, especially in the beginning of the concentration process, but these differences diminishing by the end of processing. The experiments were carried out for two hours, which corresponded to a final volumetric reduction ratio of 1.752 for the Panzym Super E treated juice, 1.344 for the Trenolin enzyme with 1 day treatment at 25 °C, 1.382 for the Trenolin enzyme treatment for 4 days at 6 °C and 1.341 for the control sample.

The highest initial flux was observed in the case of Panzym Super E-treated blackcurrant juice, followed by the samples treated with Trenolin enzyme for 4 days. The longer Trenolin enzyme treatment resulted in higher permeate flux than the 1-day treatment at 25 °C. As expected, the lowest flux was achieved with the control sample.
In order to enable comparisons, a moving average of the permeate flux was calculated for the enzyme-treated and control samples.

The Panzym Super E-treated sample had an average flux of 6.26 L/m²/h, followed by the Trenolin 4-day treatment with a value of 5.35 L/m²/h. When the Trenolin enzyme treatment was carried out for 1 day at 25 °C, the average flux in the concentration process was 3.94 L/m²/h. The control sample had the lowest flux, with a moving average value of 2.99 L/m²/h.

In Paper III, the efficiency of Panzym Super E was compared with that of Rohapect berry, an enzyme specifically developed for the treatment of berries (Figure 14). In addition, ultrafiltered and centrifuged juices were concentrated in the reverse osmosis process.
Fig. 14. Comparison of permeate flux in the reverse osmosis process using AFC 99 membrane at an operation parameters of 45 bar pressure, temperature of 30 °C and a recirculation flow rate of 800 L/h as a function of volumetric reduction factor. OJ1+PA+CE: Panzym Super E-treated and centrifuged juice, OJ2+RB+CE: Rohapect berry-treated and centrifuged juice, UFJ: ultrafiltered juice, OJ3+CE: centrifuged blackcurrant juice samples (modified from Paper III © 2010 Elsevier).

As pointed out in Paper III, the volumetric reduction factor and the total soluble solids are affecting the permeate flux, as they increase, the permeate flux drops.

Moving average of the permeate flux change gives a good possibility to compare the permeate fluxes of the different test runs. In the reverse osmosis, the juice that was ultrafiltered had the highest permeate flux with an average value of 13.1 L/m²/h under the operating conditions of 30 °C and 45 bar. This was followed by the juices that were treated with Panzym Super E enzyme and centrifuged thereafter (OJ1+PA+CE) with an average permeate flux of 11.9 L/m²/h. The use of Rohapect berry enzyme followed by a centrifugation(OJ2+RB+CE), increased the average permeate flux to 9.2 L/m²/h when compared to the centrifuged juice (OJ3+CE) that showed the lowest average permeate flux, 7.3 L/m²/h. The experiments were run until the permeate flux almost stopped, generally the concentration lasted for about 1 hour.
6.2.2 Determination of resistances using the resistance-in-series model

In order to better understand the flux behaviour of different blackcurrant juice samples in reverse osmosis, the membrane resistance, fouling resistance and gel layer resistance were calculated in m⁻¹ using the resistance-in-series model (Table 7).

Table 7. Membrane resistance (R_M), fouling resistance (R_F), gel layer resistance (R_P) and total resistance (R_T) of the blackcurrant juices during concentration by reverse osmosis (modified from Paper II © 2009 Elsevier).

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>R_M</th>
<th>R_F</th>
<th>R_P</th>
<th>R_T</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(m)</td>
<td>(m)</td>
<td>(m)</td>
<td>(m)</td>
</tr>
<tr>
<td>Control</td>
<td>2.920x10¹⁴</td>
<td>1.985x10¹³</td>
<td>3.850x10¹⁴</td>
<td>6.970x10¹⁴</td>
</tr>
<tr>
<td>PSE enzyme</td>
<td>2.920x10¹⁴</td>
<td>1.141x10¹³</td>
<td>1.964x10¹⁴</td>
<td>5.025x10¹⁴</td>
</tr>
<tr>
<td>Trenolin 4 days 6 °C</td>
<td>2.920x10¹⁴</td>
<td>1.578x10¹³</td>
<td>1.338x10¹⁴</td>
<td>4.416x10¹⁴</td>
</tr>
<tr>
<td>Trenolin 1 day 25 °C</td>
<td>2.920x10¹⁴</td>
<td>1.962x10¹³</td>
<td>2.678x10¹⁴</td>
<td>5.794x10¹⁴</td>
</tr>
</tbody>
</table>

The resistance-in-series model provided the possibility to study the reasons behind the permeate flux drop in the concentration process in more detail. The calculations revealed that the fouling resistance was one order of magnitude smaller than the membrane resistance. When comparing the total resistances between the different pre-treatments, the ranking of the samples was found to be: Control > Trenolin enzyme 1 day 25 °C > PSE enzyme > Trenolin enzyme 4 days 6 °C.

Although the Trenolin enzyme 4 days 6 °C treatment had the lowest total resistance in the modelling, the contribution of fouling resistance was much higher than in the case of Panzym Super E treatment. The Trenolin enzyme is a specific pectinase enzyme preparation with no defined side-activity. On the other hand, the Panzym Super E preparation has a defined side-activity of hemicellulose, cellulase and arabanase.

When Panzym Super E is used for pre-treatment of the blackcurrant juices, due to the side-activity of the enzyme, the natural cellulose, hemicellulose and arabanose contents of the juices will also be affected. It can be expected that the hemicellulose and cellulose contents of the juice may play a role in the extent of the fouling of the membrane, and once they are at least partially decomposed due to the enzymatic treatment, it will result in a lower fouling resistance of the membrane.
As a general rule, fouling of the membrane should always be minimised due to the fact that when fouling occurs, the process has to be stopped and the membrane cleaned or replace, which results in a direct capital loss.

6.2.3 Determination of total resistance and concentration polarisation value using the osmotic pressure model

One aim in Paper II was to prove the applicability of the osmotic pressure model to describe the total resistance and the concentration polarisation value of the concentration of four different samples of blackcurrant juice: Panzym Super E-treated, Trenolin enzyme 1 day 25 °C, Trenolin enzyme 4 days 6 °C and control samples.

In the examined case, the osmotic pressure may be caused by accumulation of glucose molecules in the boundary layer near the membrane surface. In this case, making an assumption that the total soluble solids content is mainly glucose to simplify the modelling, by plotting the molar concentration of the juice against the permeate flux, the average concentration polarisation values can be calculated from the equation of the fitted straight line, as illustrated in Figure 15. The fitted equation is described in Table 8.

Fig. 15. Permeate flux as a function of glucose concentration during concentration of blackcurrant juice samples by reverse osmosis using AFC 80 tubular membrane at a pressure of 60 bar, recirculation flow rate of 900 L/h and temperature of 25 °C (modified from Paper II © 2009 Elsevier).
The calculated values of the concentration polarisation ($\beta$) are presented in Table 8. As can be seen from the data, the highest concentration polarisation effect was observed in the control samples and the lowest in the juice treated with Panzym Super E.

Taking into account the total resistances (Table 7), the concentration polarisation value and the decreasing rate of the normalised flux (Figure 15), pretreatment of blackcurrant juice with Panzym Super E to improve the filtrability and to achieve high end-concentration of the retentate is recommended in industrial applications.

To improve the economics of concentration of blackcurrant juice using reverse osmosis, the enzyme treatment parameters, including the concentration, temperature and treatment time, should also be optimised.

Table 8. Calculated concentration polarisation values of different pre-treated juice types (Paper II © 2009 Elsevier).

<table>
<thead>
<tr>
<th>Pre-treatment</th>
<th>Fitted equation</th>
<th>$R^2$</th>
<th>$\beta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$-7.25\times10^{-6}x + 9.78\times10^{-6}$</td>
<td>0.910</td>
<td>2.035</td>
</tr>
<tr>
<td>Panzym Super E enzyme</td>
<td>$-8.21\times10^{-6}x + 1.36\times10^{-5}$</td>
<td>0.984</td>
<td>1.467</td>
</tr>
<tr>
<td>Trenolin enzyme 4 days 6 °C</td>
<td>$-1.04\times10^{6}x + 1.54\times10^{-5}$</td>
<td>0.969</td>
<td>1.627</td>
</tr>
<tr>
<td>Trenolin enzyme 1 day 25 °C</td>
<td>$-7.97\times10^{-6}x + 1.18\times10^{-5}$</td>
<td>0.972</td>
<td>1.640</td>
</tr>
</tbody>
</table>

6.2.4 Anthocyanin and flavonol content of juice and concentrate following concentration by reverse osmosis

In Paper III, the changes in anthocyanin and flavonol content were evaluated after enzyme treatment of the juices with Panzym Super E and Rohapect berry preparations, after centrifugation of the juices and after concentration by reverse osmosis. In order to evaluate the significance of the difference, analysis of variance (ANOVA) was carried out in all cases.

The second aim of enzyme treatment, in addition to ease of filtration through the decomposition of the pectin, was to increase the anthocyanin and flavonol content. The results indicated that treatment with Panzym Super E and Rohapect berry enzymes had a positive effect on the content of valuable compounds, since the increase was significant in the case of both enzymes. The statistical analysis proved that the enzyme-treated juices had significantly higher concentrations of anthocyanin ($F = 8.288, p=0.011$) and total flavonol ($F = 2.678, p=0.0129$).
The total anthocyanin content increased to a slightly higher value in the case of Rohapect berry enzyme treatment, i.e. from 884.4 to 1361.0 mg/L, while in the case of Panzym Super E it increased from 835.8 to 1295.6 mg/L. However, the difference in anthocyanin increase between the two enzyme treatments was not statistically significant.

In the Panzym Super E treatment the flavonol content rose from 42.9 mg/L to 59.7 mg/L, while in the Rohapect berry enzyme treatments from 53.1 mg/L to 77.8 mg/L.

These results indicated that the enzyme treatment process is more successful to increase the anthocyanin content of the blackcurrant juice when compared to the increase in flavonol content. The anthocyanin content increased by approximately 55%, while the increase in flavonol content was very much dependent on the enzyme used and varied between 39-46%.

This phenomena is also supported by the finding of Koponen et al. (2008a, 2008b). They investigated the effect on enzyme treatment to blackcurrant and bilberry to achieve higher anthocyanin and flavonol content. Using different enzyme preparations, they succeeded in increasing the anthocyanin content of blackcurrant juice by up to 58% and the flavonol content by 48%.

Blackcurrant juice has to undergo a clarification process prior to concentration by reverse osmosis. As an alternative to the ultrafiltration process, clarification by centrifugation was investigated. Since some of the fruit pulp present in the juice is removed during centrifugation, the anthocyanin and flavonol content may be affected. Iversen (1999) observed this effect for anthocyanins in blackcurrant juice.

The decline in the anthocyanin content was significant (F = 7.121, p=0.028) due to the centrifugation process. The decrease of 98% was observed in the non-enzyme treated sample, 90.1% in the Panzym Super E-treated sample and 75.7% in the Rohapect berry-treated sample.

Statistical analysis of the decrease in flavonol content of the juices showed that it was significant (F = 5.079, p=0.054). Similar findings were made for the flavonol content. The greatest decrease in flavonol content was found in the Rohapect berry enzyme-treated samples (74.4% preservation of flavonols), followed by the Panzym Super E treatment (97.0%), while the best results were observed for non-enzyme treated samples (97.1% preservation of flavonols).

In addition to the centrifugation process, it was also of interest to determine whether the blackcurrant juice concentrates differed in anthocyanin and flavonol content. The results indicated that the difference among the different concentrates
were significant both from the view of anthocyanins (F = 72.13, p=0.014) and flavonols (F = 14.09, p=0.001) content (Figure 16a and 16b, respectively).

Fig. 16. Effect of pre-treatment on the final a) anthocyanin and b) flavonol content in blackcurrant juice concentrates. OJ1+PA+CE: Panzym Super E-treated blackcurrant juice sample after centrifugation and concentration, OJ2+RB+CE+ROJ Rohapect berry-treated blackcurrant juice sample after centrifugation and concentration, OJ3+CE+ROJ blackcurrant juice sample after centrifugation and concentration, UFJ+ROJ ultrafiltered concentrated blackcurrant juice (modified from Paper III © 2010 Elsevier).

The lowest anthocyanin and flavonol content was found for the ultrafiltered juice concentrate (UFJ+ROJ), which contained 530.6 mg/L anthocyanins and 64.4 mg/L flavonols. The highest anthocyanin content, 1830.0 mg/L, was achieved by the Panzym Super E treatment combined with centrifugation and concentration (OJ1+PA+CE+ROJ). At the same time it contained 80.7 mg/L flavonol. The Rohapect berry-treated juice (OJ2+RB+CE+ROJ) and the centrifuged original juice (OJ3+CE+ROJ) did not differ significantly, with the anthocyanin content increasing to 1471.8 and 1338.4 mg/L, respectively. The Rohapect berry treated and concentrated juice (OJ2+RB+CE+ROJ) contained 78 mg/L flavonol while the original centrifuged juice (OJ3+CE+ROJ) had the highest flavonol content, 83 mg/L.
6.3 Extraction procedures for anthocyanin recovery from blackcurrant marc

This section introduces the significant terms in microwave-assisted extraction, i.e. process parameters determined with the use of response surface methodology, in order of importance and presents the results on optimisation of these processing parameters. The optimum process parameters were plotted in a contour plot and the optimum values for the microwave power and extraction time were determined. In addition, anthocyanin extraction by conventional solvent was evaluated using three different solvents. The two methods were then compared in terms of the anthocyanin yield and the effect of the processing method on the anthocyanin composition was evaluated.

6.3.1 Optimum processing parameters in microwave-assisted extraction

A polynomial equation was used to fit the experimental data to the yield of anthocyanins. In the next step, the process parameters that had no significant effect on the anthocyanin yield were removed from the model. The resulting regression coefficients were as follows: $R^2 = 0.930$, $R^2_{adj} = 0.911$, $Q^2 = 0.853$ and reproducibility = 0.9337. The model was also tested for accuracy by ANOVA, which showed that the model was statistically significant ($p<0.05$) and no lack of fit was observed ($F = 1.4136$, $p=0.403$). Based on this, the model was deemed adequate to predict the optimum processing parameters.

Based on the ANOVA results, the most important and significant terms were investigated and are illustrated in Figure 17. The coefficients of linear terms, i.e. the microwave power ($X_1$), the marc to solvent ratio ($X_2$), the pH of the solvent ($X_3$) and the extraction time ($X_4$), had a significant effect on the amount of anthocyanins in the extracts. The interaction terms of the microwave power with the extraction time ($X_1*X_4$) and the pH of the solvent with the extraction time ($X_3*X_4$) also made significant contributions to the maximum recovery of anthocyanins in microwave-assisted extraction of blackcurrant marc.

The importance of process variables was as follows: microwave power ($X_1$) > microwave power with extraction time interaction term ($X_1*X_4$) > extraction time ($X_4$) > the pH and extraction time interaction effect ($X_3*X_4$) > pH of the solution ($X_3$) > marc to solvent ratio linear term ($X_2$). A similar ranking was found by Sun et al. (2007), who concluded that the microwave power and the extraction time
were the most important variables in the recovery of anthocyanins from red raspberries in the microwave-assisted extraction process, while the effect of solvent to solid ratio was less significant.

Fig. 17. Importance of process variables in microwave-assisted extraction of anthocyanins from blackcurrant marc. MWP = microwave power (W), MWP*t = microwave power and extraction time interaction term, t = extraction time, pH*t = pH of the solvent and extraction time interaction term, pH = pH of the solvent, S:L = marc to solvent mass ratio (Paper IV © 2013 Springer).

The polynomial equation obtained based on Equation 19 was:

\[ Y = 18.714 + 2.99778X_1 - 0.608X_2 - 0.73916X_3 + 2.15806X_4 \\
- 2.195X_1X_4 + 0.8331X_3X_4 \]

Based on the constructed mathematical model, it proved possible to optimise the process parameters. This optimisation was carried out as follows: the pH was fixed at pH 2, since anthocyanins show a more stable red colour form at this pH, and a solid to liquid ratio of 0.05, which means the highest amount of water added to the marc in order to accelerate the extraction process. The results are illustrated in the form of contour plot in Figure 18.
Fig. 18. Response surface plot of anthocyanin extraction from blackcurrant marc by microwave-assisted extraction. MWP = microwave power (W), Time = extraction time in minutes, MAP = monomeric anthocyanin pigments as mg/g cya-3-glu equivalents (Paper IV © 2013 Springer).

The marc to solvent ratio influences the driving force during mass transfer (Pinelo et al., 2005) and thus reduces the extraction time. The chemical form, and thereby the colour of anthocyanins, depends on the pH of the solution (Castaneda-Ovando et al., 2009).

Low pH favours the stability of anthocyanins. Cabrita et al. (2000) reported that in very acidic aqueous solutions, the anthocyanin 3-glucosides occur in the most reddish colour typical of their flavylium form, and in their study showed stability of over 70% after 60 days storage at 10 °C.

The optimisation process showed that the higher the microwave power, the shorter the extraction time needed to reach a high anthocyanin yield as was shown within the experimental condition ranges investigated in this thesis. The optimum process variables for maximum recovery of anthocyanins from blackcurrant marc
were determined as a microwave power of 700 W and an extraction time of 10 min.

At the end of the optimisation process, it was necessary to verify the accuracy of the constructed model by repeating the experiments at the optimum process parameters. The results from the validation process showed that the values obtained for the anthocyanin content in the duplicate repeat experiments were 24.0 ± 0.0 mg/g and 25.1 ± 0.1 mg/g, which were close to the value of 23.9 mg/g predicted in the optimisation process. This means that the optimum process parameters were successfully identified.

6.3.2 Conventional extraction of anthocyanins from blackcurrant marc

The efficiency of the microwave-assisted extraction procedure was compared with that of conventional solvent extraction using different solvents at pH 2, the same pH at which the microwave-assisted extraction was carried out. The solvents studied were aqueous hydrochloric acid, citric acid and citric acid combined with sulphur dioxide. These solvents were chosen based on the results of our preliminary research carried out previously on the utilization of berry processing by-products and they rely on unpublished data.

Temperature of extraction was 80 °C, which is almost identical to that measured by an infrared thermometer at the end of the microwave-assisted extraction under optimum processing parameters.

The results from the solvent extraction indicated that the rise in monomeric anthocyanin pigments was much higher in the first three hours of the extraction process, and tended to slow down towards the end of the five-hour extraction period (Figure 19). The most probable reason for this lies in the temperature sensitivity of the anthocyanins, which tended to degrade during the prolonged exposure time at the 80 °C extraction temperature.

The efficiency of solvent extraction was highly dependent on the solvent used. The highest concentration of anthocyanins was achieved when the aqueous hydrochloric acid was used at pH 2, which is the same solvent as was used in the microwave-assisted extraction. This provided good possibilities to make a comparison between the two extraction processes from the point of view of the efficiency. Since the microwave-assisted extraction can be considered as a rapid process, opposite to the conventional extraction, and therefore no significant anthocyanin degradation is expected, samples for analyses were not collected
continuously during the 10 minutes irradiation time. Although, this could be possible by improving the process design of the equipment to make the sample taking easier, in this work, the comparison was made based on the anthocyanin content of the final extract with the extract from conventional extraction at 1, 2, 3, 4, and 5 hours treatment time.

![Graph showing anthocyanin yield as a function of time in conventional solvent extraction at 80 °C of temperature for 5 hours using an Armfield solvent extractor.](modified_from_Paper_IV_©_2013_Springer)

**Fig. 19.** Anthocyanin yield as a function of time in conventional solvent extraction at 80 °C of temperature for 5 hours using an Armfield solvent extractor (modified from Paper IV © 2013 Springer).

### 6.3.3 Comparison of microwave-assisted extraction and conventional solvent extraction

In order to evaluate the two alternative extraction processes, a comparison was made in terms of efficiency of anthocyanin recovery from the blackcurrant marc. To this end, the analysis results of the extracts obtained at the optimum processing conditions in microwave-assisted extraction was compared to the analysis results of the conventional extraction samples obtained at different time of the extraction using solvents of different nature. During the rapid microwave-assisted
extraction, no samples were collected meanwhile, but the anthocyanin content of the end product was determined.

The results indicated that in the microwave-assisted extraction, the temperature raised rapidly to reach 69.7 °C, which is near to that temperature used also in the conventional extraction process (80 °C). Although, not analysed specifically how the anthocyanin content varied during the 10 minute extraction process using a microwave power of 700 W, it can be expected that due to the shortness of the treatment, no significant degradation of anthocyanins would occur. In contrast to this, the analyses from the samples during the conventional extraction in 1-5 hours of extraction time showed that after the initial rise in the anthocyanin content, it begin to drop by the end of the time frame under investigation which may refer to a degradation process.

From the determination of the anthocyanin content of the samples, it can be concluded that using the microwave power of 700 W for 10 minutes time to extract anthocyanins using aqueous HCl as a solvent, the anthocyanin concentration of the end product was 24.0 ± 0.0 mg/g and 25.1 ± 0.1 mg/g. This is approximately 20% higher than the anthocyanin concentration of 16.7 mg/g that was reached as the maximum value in a conventional extraction using an aqueous HCl solvent as well.

Although an economic evaluation of the two processes was not done in the frame of the thesis, it can be concluded that by implementation of microwave-assisted extraction, the anthocyanin recovery becomes also faster, since in this process, the extraction was carried out for 10 minutes, and in contrast, in the conventional extraction, the maximum value was obtained in a test that was carried out for 300 minutes.

**6.3.4 Quality of the extracts**

In the following it is investigated whether the processing altered the anthocyanin profile of the extracts in terms of composition. To this end, detailed analysis of the samples was carried out using the HPLC method.

The anthocyanin composition of three samples from the conventional extraction after 300 minutes of extraction time and of four randomly chosen samples from the microwave-assisted extraction, including the processing at optimal parameters, were determined by HPLC as described before and the most abundant anthocyanins of delphinidin-3-glucoside (dp-3-glu), delphinidin-3-rutinoside (dp-3-rut), cyanidin-3-glucoside (cya-3-glu), cyanidin-3-rutinoside
(cya-3-rut) and malvidin-3-rutinoside (mal-3-rut) were calculated. The results are summarised in Table 9.

Table 9. Composition of the anthocyanin extracts in microwave-assisted and conventional extraction determined by HPLC. CE = conventional extraction, CA = citric acid, HCl = hydrochloric acid, SO₂ = sulphur dioxide, MAE = microwave-assisted extraction, MAE* = microwave-assisted extraction under optimised process conditions, MWP = microwave power (Paper IV © 2013 Springer).

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>MWP (W)</th>
<th>Time (min)</th>
<th>pH</th>
<th>Delphinidin-3-glucoside (mg/g) (%)</th>
<th>Delphinidin-3-rutinoside (mg/g) (%)</th>
<th>Cyanidin-3-glucoside (mg/g) (%)</th>
<th>Cyanidin-3-rutinoside (mg/g) (%)</th>
<th>Malvidin-3-rutinoside (mg/g) (%)</th>
<th>Total (mg/g) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE+CA</td>
<td>-</td>
<td>300</td>
<td>2</td>
<td>3.2±0.1 25.4 4.7±0.1 37.5 1.2±0.0 9.5</td>
<td>3.3±0.1 26.4 0.2±0.0 1.2 12.5±0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE+HCl</td>
<td>-</td>
<td>300</td>
<td>2</td>
<td>4.0±0.2 24.4 6.2±0.2 37.5 1.6±0.1 9.7</td>
<td>4.6±0.1 27.9 0.1±0.0 0.5 16.7±0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CE+CA+SO₂</td>
<td>-</td>
<td>300</td>
<td>2</td>
<td>2.7±0.2 22.1 5.5±1.0 44.6 0.9±0.0 6.9</td>
<td>3.1±0.1 25.1 0.2±0.0 1.3 12.2±1.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAE</td>
<td>140</td>
<td>10</td>
<td>2</td>
<td>4.5±0.3 27.9 5.5±0.2 39.8 1.0±0.0 7.3</td>
<td>3.3±0.1 23.9 0.1±0.0 1.0 13.7±0.5</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>MAE</td>
<td>140</td>
<td>10</td>
<td>2</td>
<td>2.7±0.1 28.1 3.9±0.1 40.1 0.7±0.0 7.2</td>
<td>2.3±0.0 23.7 0.1±0.0 0.9 9.7±0.3</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>MAE</td>
<td>140</td>
<td>30</td>
<td>2</td>
<td>3.4±0.1 20.2 7.5±0.3 44.3 1.2±0.0 7.3</td>
<td>4.5±0.0 26.9 0.2±0.0 1.4 16.9±0.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAE*</td>
<td>700</td>
<td>10</td>
<td>2</td>
<td>5.6±0.3 27.5 8.4±0.2 41.2 1.5±0.1 7.1</td>
<td>4.8±0.1 23.3 0.2±0.0 0.9 20.4±0.8</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

The most abundant anthocyanin compound was dp-3-rut, with a relative distribution of 37.5-47.1% in different extracts, followed by cya-3-rut (23.7-27.9%) and dp-3-glu (18.5-28.3%), while cya-3-glu and mal-3-rut were present in all extracts at much lower concentrations, between 5.8-9.7% and 0.5-1.4%, respectively.

The results of the analysis showed that the composition of the extracts was similar to that reported in the literature, e.g. Mattila et al. (2011) and Landbo & Meyer (2004) reported a similar distribution of the individual anthocyanins for blackcurrants. Iversen (1999) found a similar composition in both blackcurrant berries and juice.

After analysing the samples, it was confirmed that the extraction processes were not selective for any of the compounds, with the extracts having the same composition as the original raw material, blackcurrant berries. Comparing microwave-assisted extraction with conventional extraction, it can be concluded that the microwave treatment only accelerated the rate of the extraction, and did not compromise the composition of the extracts.
7 Summary and conclusions

In this thesis work, the main focus was on the development of technologies to prevent degradation of valuable components and generation of food waste in the processing of blackcurrants.

To this end, membrane technologies, including ultrafiltration and reverse osmosis, were implemented into juice concentrate production with the aim of concentrating the juice in gentle processing conditions, since the valuable compounds in blackcurrants are sensitive to heat and processing.

Juice samples were first depectinised using a commercial enzyme preparation in order to enhance the filtration efficiency, measured as permeate flux. For this purpose, different commercially available pectinase enzyme preparations were used that had diverse side activities. An optimal enzyme concentration of the Panzym Super E, Trenolin and Rohapect berry products were determined. Since the valuable compounds are sensitive to processing, the amounts of anthocyanins and flavonols were measured during the filtration process.

The results indicated that ultrafiltration affected retention of these compounds in the clarified juice. Only 50% of the total anthocyanins and 46% of the total flavonols were available in the ultrafiltered juice compared with the original raw material. During the ultrafiltration, both the technical and operational parameters will determine to what extent these compounds are retained or passed by the membrane. The gel layer built up on the membrane surface as the filtration process continues could also affect the rejection of anthocyanins and flavonols largely. Moreover, phenolic compounds tend to form complexes with proteins existing in the blackcurrant juice, and their bigger molecular weight, complex formation could be a reason for such analytical results.

The major role of ultrafiltration is to clarify the blackcurrant juice and therefore increase the efficiency of the reverse osmosis concentration process. For the ultrafiltration process, Pellicon 2 module equipped with a Biomax membrane with an active membrane area of 0.1 m², with a nominal weight cut-off value (NWCO) of 100 kDa was used.

The reverse osmosis experiments were carried out with equipment that contained a B1 tubular module. The module was equipped with AFC 80 or AFC 99 membranes made from polyamide film, indicating the NaCl rejection values of the membranes at the maximum pressures allowed to use for the membranes. The results indicated that the ultrafiltered juice had the highest permeate flux, but application of enzymatic depectinisation combined with centrifugation was also a
promising method to increase the concentration efficiency. Since ultrafiltration decreased the availability of the valuable compounds in the blackcurrant juice, it is recommended that the clarification process be replaced with enzymatic depectinisation, to enhance the permeate flux during reverse osmosis. This recommendation is supported by the amounts of anthocyanins and flavonols in the final concentrates, since the enzyme-treated blackcurrant juices tended to have higher amounts of valuable compounds after concentration.

In the processing of blackcurrant juice, it is essential that the compounds that are beneficial for health are maintained in the final product. The efficiency of the concentration process in reverse osmosis was described by the terms of the moving average of the permeate fluxes. From this point of view, the ultrafiltered juice had the highest permeate flux with an average value of 13.1 L/m²/h under the operating conditions of 30 °C and 45 bar. Despite of this, the retention of the beneficial compounds was significant, and therefore it should be avoided as a pre-treatment method.

The ultrafiltered juice was followed by the Panzym Super E enzyme and centrifuged juice (OJ1+PA+CE) with an average permeate flux of 11.9 L/m²/h. The use of the Rohapect berry enzyme followed by a centrifugation (OJ2+RB+CE), increased the average permeate flux to 9.2 L/m²/h when compared to the centrifuged juice (OJ3+CE) that showed the lowest average permeate flux, 7.3 L/m²/h. These results suggests that the effect of the centrifugation process on the permeate flux is enhanced by combining it with the pectinase enzyme treatments. As a rule, to ensure the good filterability, economy of the process and the shelf life of the membrane however, the centrifugation as a pre-purification process of the juice should not be omitted.

In blackcurrant pressing, the marc, stays behind as a by-product of processing. The current practice in small and medium-sized companies is to utilise this by-product as a fertiliser or animal feed. However, the marc contains the skins, the marc and the seeds of the blackcurrants and can be valorised by extracting anthocyanins in a microwave-assisted extraction process. This thesis showed that utilisation of by-products by proper technologies could benefit processing companies by creating more valuable final products than feeds or fertilisers. For example, it could increase utilisation in the food industry of products that have high anthocyanin and pectin contents, which can be obtained in a more economically sound way by using novel extraction technologies but not by conventional extraction methods. The benefit of the novel extraction technologies is in the intensification, by using fewer resources without
compromising the product quality. Therefore, processing of the by-products may also have a positive effect on the economics of berry processing, since it results in additional products that have higher economic value.

The 10 research questions relating to value-added processing of blackcurrant berries (see section 1.1) and the scientific gaps that still exist, which were investigated in Papers I-IV, are discussed separately below.

**7.1 Answers to the research questions**

1. **How can the valuable components in blackcurrants be best preserved during the processing?**

The valuable components in blackcurrants examined in this thesis were anthocyanins and flavonols and the aim of process design was to ensure that these compounds are best preserved during the processing and are available at a high rate to provide the future consumers with healthy and nutritious end products. The literature reports that these compounds are sensitive to heat and processing, and therefore it is important that minimal and gentle processing techniques are used.

Enzyme treatment of berries is usually a long (1.5-2 hours) process, in which the berries are kept at 45 °C. It was shown here that some pectinases can work at refrigeration temperature and, although the process is slower, the valuable compounds in berries are better preserved. Once enzymatic depectinisation is carried out, the enzyme then must be inactivated at temperatures over 60 °C. Although this seems a relatively high temperature, the overall efficiency of enzymatic depectinisation in terms of elevated anthocyanin and flavonol content in the juice was demonstrated here.

The other possibility for inactivation of enzymes lies in the ultrafiltration process, in which the enzyme is retained by the membrane and the clarified juice is drained as the stream that passes through the membrane. This process can be carried out at room temperature.

If reverse osmosis is used to concentrate blackcurrant juice, some pre-treatment of the blackcurrants is required. Ultrafiltration may have a negative impact on the anthocyanin and flavonol content of the juice, as also found for clarification, and some soluble solids that contain anthocyanins and flavonols are removed. As an alternative, centrifugation is a promising method that also leads to clarification of the juice.
Once the juice has been concentrated, it is important to ensure that the microbiological quality meets relevant standards and that the shelf-life of the juice is sufficient. Pasteurisation is usually carried out for this purpose. Pasteurisation may be a high temperature, short time (HTST) or a low temperature, long time (LTLT) treatment. If the temperature used is high (e.g. 72 °C in the case of milk), the pasteurisation method lasts only for 15 seconds and is believed not to have any negative impact on the anthocyanin and flavonol content of the treated product.

In treatment of the marc, an extraction process is used to recover the anthocyanins. The thesis investigated conventional solvent- and microwave assisted extractions. The conventional extraction required not only high processing temperatures (around 80 °C), but also long treatment times, which have an adverse effect on the amount and quality of the anthocyanins in the extract. Although the temperature may also rise in microwave-assisted extraction, the treatment time is substantially lower than in the conventional extraction process described as minutes vs. hours, respectively, which allowed the maintenance of a good level of anthocyanins in the final extracts. Optimisation of the process is rather important for maximum recovery of anthocyanins.

There is a further possibility to extend the processing of the juice concentrate after reverse osmosis by membrane distillation/osmotic distillation. Moreover, the seeds of blackcurrants are a valuable source of oil, which can be extracted in a supercritical fluid extraction process. However, these processes beyond the scope of this thesis.

2. What is the role of temperature in the initial pectinase treatment of blackcurrants?

Enzymatic depectinisation takes a shorter time when higher temperature is used. For example, juice depectinisation requires 1-2 hours at 45-55 °C in the case of the Panzym Super E liquid. However, juice treatment can also be carried out at 15-25 °C for 4-8 hours. At lower temperatures than this, the reaction rate decreases and the amount of enzyme used must be increased.

In the case of Trenolin enzyme, the experiments were carried out both at room temperature for 1 day and at refrigeration (6 °C) temperature for 4 days. Tests on the efficiency of filtration in terms of permeate flux comparison revealed that the enzyme treatment carried out at 6 °C for 4 days was almost as effective as the depectinisation carried out at room temperature. Lower temperatures for depectinization are prioritized both from the process economy point of view and
due to the heat sensitivity of these compounds. However, the lowering of the processing temperature may have an adverse effect on the amount of the enzyme that needs to be used to achieve the complete depectinization of the juices. This may have a greater impact on the process economy. The cost of enzymes are usually high and for this reason it shall be taken into account in the decision making process.

3. What is the impact of enzyme dosage on the permeate flux in the blackcurrant juice clarification process?

The filtration efficiency was described by the permeate flux during clarification by ultrafiltration. It was established that enzymatic depectinisation had an effect on the permeate flux, i.e. the higher the enzyme dosage, the higher the permeate flux.

However, increasing the enzyme level above 250 mL/100 L juice gave no significant further rise in the permeate flux compared with a dose of 200 mL/100 L juice. This suggests that there is a maximum concentration that should be used in order to run the process economically, since the cost of the enzyme has a direct effect on the profitability of processing.

4. Does the ultrafiltration alter the valuable compounds content of filtered blackcurrant juice?

Ultrafiltration was used as an alternative method to conventional juice clarification. In conventional clarification, fining agents such as bentonite, gelatin and silica sol may be used to achieve stability of the juice and to avoid haze formation and sedimentation. In the ultrafiltration process it is possible that protein-phenolic complexes are formed and due to the increased molecular weight of the complex they will be retained by the membrane and the clarified juice passes through the membrane. With these complexes, some of the anthocyanins and flavonols present are also removed.

In this thesis, an ultrafiltration membrane of 100 kDa made of polyethersulfone was used and the results indicated that the ultrafiltration process had a significant effect on the anthocyanin and flavonol content of the juices. On average, only 54% of total flavonols and 50% of total anthocyanins were retained in the ultrafiltered juice compared with the raw material.
5. Is ultrafiltration of blackcurrant juice prior to concentration by reverse osmosis necessary?

The juices were compared in the reverse osmosis process based on their filtration efficiency. In these comparisons, clarification of the juice by ultrafiltration, centrifugation and enzymatic depectinisation was investigated.

The results indicated that the highest efficiency, expressed as the permeate flux, was achieved by the previously ultrafiltered juice, followed by the enzyme-treated juices after centrifugation. The lowest permeate flux was observed when the juices were clarified only by centrifugation.

It is important to observe that, between the two clarification methods, i.e. ultrafiltration and centrifugation, the average permeate flux was almost doubled in the case of the ultrafiltered juice compared with the centrifuged juice.

Ultrafiltration is recommended not only because it results in a higher permeate flux in concentration by reverse osmosis, but also because it achieves complete clarification of the juice and possible removal of the haze-forming protein-phenolic complexes, and therefore the quality of the juice is enhanced.

However, since it also has a significant effect on the anthocyanin and flavonol content in the juice (Paper I), alternative clarification processes should be sought instead of ultrafiltration. Since the permeate flux in the reverse osmosis concentration of the enzyme-treated juices was comparable to that of the ultrafiltered juice, this clarification method is recommended instead of ultrafiltration.

6. Which are the most important resistances causing a drop in permeate flux when concentrating blackcurrant juice by reverse osmosis?

The resistance-in-series model made it possible to determine the total resistance, membrane resistance, fouling resistance and polarisation resistance in the process of concentrating juices by reverse osmosis.

The modelling results indicated that the highest total resistance occurred with the control juice sample, which accordingly had the lowest flux during concentration. This was followed by the 1-day long Trenolin enzyme-treated and the Panzym Super E-treated juices, while the 4-day long Trenolin treatment had the lowest total resistance.

Total resistance is the sum of the membrane, fouling and polarisation resistances. The membrane resistance was estimated to be the same in all
concentration processes, since the membranes were cleaned properly after every test run.

For all samples, the membrane and polarisation resistances were of the same order of magnitude ($10^{14}$ /m), while the fouling resistance was one order of magnitude lower for all juice samples ($10^{13}$ /m). When concentration polarization occurs, due to the high rejection of the solutes at the membrane surface, osmotic pressure will develop, which in turn will reduce the effective operation pressure ($\Delta P - \Delta \pi$). The osmotic pressure of the different juice samples can be estimated by using the van’t Hoff equation described in Equation (10).

The results indicated that osmotic pressure at the 25 °C operation temperature was 29.75 bar in the case of the control sample, 37.18 bar for the Panzym Super E treated sample, 32.22 bar for Trenolin 4 days treated sample and 33.71 bar for the Trenolin 1 day treated sample. The flux decline could be caused also by the compaction of the membrane in reverse osmosis due to the high applied pressures, which can collapse the porous structure of the membrane. The compaction of the membrane would result in irreversible deformation where the flux does not return to the previous value after the pressure is released. This was, however, not observed in these concentration processes.

7. How does reverse osmosis and subsequent processes perform in concentrating anthocyanins and flavonols in blackcurrant juice?

The initial anthocyanin and flavonol content was compared with the content at the end of the reverse osmosis process, taking into account process steps such as enzyme treatment and centrifugation of the juices.

The results indicated that the enzyme treatment increased the anthocyanin and flavonol content, while the centrifugation had a negative effect.

The highest concentrations of anthocyanins and flavonols were observed when the juices were treated with the Panzym Super E enzyme, followed by centrifugation as the clarification step and concentration by reverse osmosis. In that case, the final concentrate contained 2.19-fold more anthocyanins and 1.88-fold more flavonols than the initial blackcurrant juice.

Following the same processing scheme, the Rohapect berry-treated juice concentrate had 1.66-fold more anthocyanins and 1.47-fold more flavonols than the initial juice.

When the clarification was carried out by centrifugation only, the concentrate was found to have 1.38-fold more anthocyanins and 1.28-fold more flavonols.
Finally, the juice clarified by ultrafiltration had 1.59-fold more anthocyanins and flavonols after the reverse osmosis process.

In addition to the total increase in anthocyanins and flavonols in the concentration by reverse osmosis and subsequent processes, this thesis examined whether the process is selective for some of the individual anthocyanin and flavonol compounds. A detailed analysis was carried out to determine whether the relative ratios of dp-3-glu, dp-3-rut, cya-3-glu and cya-3-rut compounds (anthocyanins) and myricetin and quercetin (flavonols) varied in the concentration process.

It was concluded that the process was not selective for different anthocyanins or flavonols, as the final concentrate had an average composition similar to that of the initial blackcurrant juice.

8. Does microwave-assisted extraction perform better than conventional extraction in extraction of anthocyanins from blackcurrant marc?

In order to compare microwave-assisted extraction with conventional solvent extraction, a more detailed study was conducted on the latter technology. First, different solvents were analysed for their efficiency in extracting anthocyanins from blackcurrant marc. Hydrochloric acid at pH 2, citric acid at pH 2, and sulphur dioxide with 1% citric acid at pH 2 were analysed during an extraction time of 300 minutes.

The highest anthocyanin concentration (16.7 mg cya-3-glu equivalents/g) was achieved when hydrochloric acid was used as the solvent at pH 2 for 300 minutes. In comparison, with microwave-assisted extraction a maximum total anthocyanin value of 20.4 mg/g was measured after 10 minutes of irradiation using a microwave power of 700 W and the solid to solvent ratio of 0.05.

In addition to total anthocyanins, this thesis investigated whether extraction was selective for the dominant anthocyanins present in blackcurrant berries (dp-3-rut, cya-3-rut, dp-3-glu and cya-3-glu). The results of the analyses confirmed that the composition of the extracts was similar to that of blackcurrant berries reported in the literature. Therefore, method of extraction does not compromise the abundance of different anthocyanins in the final product.

In summary, it can be concluded that microwave-assisted extraction only differs by increasing the efficiency of extraction, by reducing the extraction time from 300 minutes to 10 minutes, compared with solvent extraction.
9. What are the optimum processing parameters in microwave-assisted extraction of anthocyanins from blackcurrant marc?

In order to determine the optimum processing parameters in microwave-assisted extraction, 29 test runs were conducted and the amount of anthocyanins in the extracts was recorded. A polynomial equation was fitted to the test data and, with the help of the model, the importance of different processing parameters was evaluated.

The results indicated that the coefficients of linear terms such as microwave power, marc to solvent ratio, pH of the solvent and extraction time have a significant effect on the amount of anthocyanins in the extracts. The use of the response surface modelling permitted interaction effects to be considered, among which microwave power with extraction time and pH of the solvent with the extraction time were significant. The order of importance was as follows: microwave power > microwave power with extraction time interaction term > extraction time > pH and extraction time interaction terms > pH > marc to solvent ratio.

The optimum processing parameters were then determined by keeping the marc to solvent ratio at a low level, i.e. to one unit of marc, 20 units of water were added. The higher the solvent fraction, the higher the driving force during the extraction process. The pH was kept at pH 2, since at low acidic pH the anthocyanin glucosides show their most reddish colour and are more stable.

Based on these assumptions, a microwave power of 700 W and an extraction time of 10 minutes were evaluated by the software to achieve a high anthocyanin content. The validity of the model was confirmed by repeating the experiments with the chosen process parameters, and similar anthocyanin concentrations were observed, which proves the accuracy of the model.

10. What is the best technology to achieve value-added processing of blackcurrants?

In order to benefit from the valuable compounds in blackcurrants, such are anthocyanins and flavonols, the processing technologies should be minimal and gentle.

This thesis suggested a new processing scheme that is based on integration of different membrane and extraction technologies, complemented with enzyme treatments of the berries to gain higher juice yields and enzymatic depectinisation of the juice to give higher filtration efficiency. The main aim of value-added
processing is to produce a value-enhanced juice concentrate that is rich in anthocyanins and flavonols, and to utilise the by-products from juice pressing (marc) to extract anthocyanins. The process concept with the recommended technological solutions including elements also that, although were not investigated in this thesis, but are relevant for the maximum efficiency of the blackcurrant processing (final concentration by membrane or osmotic distillation, the oil recovery and the pasteurization), is illustrated in Figure 20.

Fig. 20. Technological solution for the value-added processing of blackcurrants. Comparison of the traditional processing with the novel concept.

In the first step of juice concentrate production, the juice undergoes an enzymatic depectinisation process to degrade the pectin in the juice, which may otherwise cause fouling of the reverse osmosis membrane.

This thesis investigated whether use of higher temperatures for enzyme treatment can be avoided by extending the depectinisation time at lower (refrigeration) temperatures. This change in processing should lead to avoidance of anthocyanin degradation, which can occur due to the effect of temperature. The mash should be heated to 60 °C for complete inactivation, after which the juice can be pressed. In order to increase the efficiency of the reverse osmosis process,
the juices are depectinised by pectinase enzymes, which also enables determination of the anthocyanins and flavonols in the juice.

After depectinisation, the enzyme is inactivated by heating to 60 °C and the mixture is centrifuged to clarify the juice prior to concentration by reverse osmosis. The aim of reverse osmosis is to produce a pre-concentrate that has a total soluble solids content of 25 °Brix and to concentrate the anthocyanins and flavonols present into the juice.

A final concentrate with approximately 65 °Brix can be produced by osmotic distillation or membrane distillation, but these techniques were beyond the scope of this thesis.

Finally, the juice concentrate has to be pasteurised to ensure the microbiological quality, which is a critical step from the point of view of the anthocyanin content, since anthocyanins can easily degrade at high temperatures. As a promising option, a high temperature, low time (HTLT) pasteurisation process is recommended for a further study that may give a possibility to ensure the good quality of the end-product.

The other requirement for value-added blackcurrant processing is utilisation of the marc, which is an unavoidable by-product of juice pressing. This thesis evaluated microwave-assisted extraction of the anthocyanins from blackcurrant marc, a method which proved to be more efficient than the conventional solvent extraction method when the process parameters were optimised. These extracts should undergo purification, after which they are ready for use as food colorants and medical or cosmetic substances. If the seeds are separated in an early stage of marc processing, valuable blackcurrant oil could also be obtained in the supercritical fluid extraction process. However, that process was also beyond the scope of this thesis.

### 7.2 Further work

A novel processing method for value-added processing of blackcurrants into juice concentrates and anthocyanin extracts was devised in this thesis. The main technologies investigated included ultrafiltration and enzymatic treatments as pre-treatment methods, reverse osmosis for juice concentration and microwave-assisted extraction of anthocyanins.

To analyse the high retention of anthocyanins and flavonols during the ultrafiltration, supplementary studies are required whether the phenomenon can be described by protein-phenolic complex formation during the processing, which
complexes due to the increased molecular weight of the compound will be retained by the 100 kDa cut-off value membrane. It is possible that in addition to the naturally occurring proteins of the blackcurrant juice, the pectinase enzymes added to the juice during processing will also follow this. This information and a proof by analysis methods would be essential for the evaluation of the UF process from the point of view of viability. The reverse osmosis process is a viable method to prepare a pre-concentrate, but further experiments should be carried out to prove the applicability of osmotic/membrane distillation for final concentration of the juice.

In the present research, ensuring and investigating the microbiological quality and shelf-life of the juice concentrates was excluded. From the food safety point of view, such investigations should be carried out to ensure that the juice provided to consumers is safe to drink.

It would be valuable to get feedback from consumers or results from trained tasters on the organoleptic properties of the juice concentrates and the acceptability to consumers. Marketing research should be carried out to evaluate whether consumers are interested in value-added juice concentrates and willing to pay more for such products.

The processing of blackcurrant marc can be extended by recovery of the blackcurrant seed oil, which should be of interest to the cosmetics industry.

In order to evaluate the viability of a new business concept, the economic viability of concentration and extraction processes should be calculated. This would provide the necessary information about the profitability of the process for berry processing companies.

Finally, the sustainability of the process model developed should be studied. This would not only raise awareness among berry processing companies about environmentally friendly processing, but would also help environment-conscious consumers in making their choices to purchase the new value-added products.
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The thesis is based on the following original research papers:


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VALUE-ADDED PROCESSING OF BLACKCURRANTS

USE OF MEMBRANE TECHNOLOGIES FOR CLARIFICATION AND CONCENTRATION OF BLACKCURRANT JUICE AND EXTRACTION OF ANTHOCYANINS FROM BLACKCURRANT MARC