

CHALMERS



Effects of heat treatment and additives on the anthocyanin content in
blackcurrants and its relation to colour and texture

Master of Science Thesis in the Master Degree Programme, Biotechnology

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Abstract

Over the last decade there has been a growing interest in the potential health benefits from eating a diet rich in fruit and berries containing large amounts flavonoids. Among these the anthocyanins have been given a lot of attention and studies indicate that they might help prevent tumour growth and urinary tract infections to mention a few of the potential benefits. In studies on model systems, anthocyanins have been proven to be degraded by heat, oxygen access, pH increases and addition of sugar.

In this study the focus has been shifted to the effects of heat and other common processing parameters on anthocyanins in a blackcurrant mash, which is more resembling to real products. Anthocyanins were extracted from blackcurrants by using acidified methanol solution. UV-spectrophotometry was used to measure the anthocyanin concentrations. The effect on colour and texture was also investigated by colourimetry with a hand held coulormeter and shear rate ramp tests with a rheometer respectively.

The results indicate that it is possible to predict anthocyanin degradation in blackcurrant mash induced by heating with a simple exponential decay model. Additionally it was concluded that the pH level is a major factor influencing the degradation speed. Furthermore, an increase in the glucose and ascorbic acid content decrease and increase the anthocyanin degradation respectively. It was also concluded that the colour is affected by the anthocyanin degradation but no clear correlation was found. Eventually no correlation between anthocyanin content and texture was found.

Sammanfattning

Mat och hälsa är ständigt aktuella ämnen i människors vardagsliv. En snabb koll på några av de stora dags- och kvällstidningarnas hemsidor eller ett besök vid tidningsstället i en närliggande mataffär bekräftar detta. Den nuvarande trenden går också mot mer gammaldags produkter som uppfattas som ett hälsosamt alternativ till de processade och bearbetade livsmedlen från industrin. Bär och frukt tillhör de livsmedel som på senare tid har blivit mer och mer uppmärksammas för dess påstådda hälsofördelar och association till vad som uppfattas som en sund livsstil.

Studier under de senaste decennierna har därför försökt verifiera dessa påståenden och undersöka vilka ämnen i bär och frukter som ger den positiva hälsoeffekten. Man har funnit att ämnesgruppen antocyaniner, som ger de flesta bär och frukter dess blåa och röda färger, kan ligga bakom denna effekt. Studier har visat att antocyaniner kan hjälpa mot bland annat urinvägsinfektioner, hjärt- kärlsjukdomar och hämma tumörtillväxt. Andra studier på modellsystem har dock visat att anthocyaninerna bryts ner av behandlingar som är vanliga inom livsmedelstillverkning såsom uppvärmning, tillgång till syre och tillsättning av askorbinsyra. Den här studien har därför ägnats åt att studera huruvida detta sker även i mer matlika produkter såsom svartvinbärsmos och puré och undersökt hur fort detta sker. Då antocyaninerna är det som ger upphov till de blå, lila och röda färgerna i svarta vinbär och blåbär har även påverkan på färgen undersökt och om den går att korrelera till antocyaninhalten. Slutligen har påverkan av värme och ett antal ingredienser på konsistensen undersökts då svarta vinbär innehåller höga halter av pektin.

Värmebehandling av svartvinbärsmos visade att antocyaninerna sönderfaller exponentiellt och att temperaturen var den viktigaste faktorn. Jämförelser mellan svarta vinbär och blåbär visade att vid likvärdiga förhållanden minskar halten antocyanin i blåbär fortare än i svarta vinbär. Detta förmodligen beroende på att de olika antocyaninerna är olika värmetåliga. När bärsorterna blandades så bröts antocyaninerna ner lika fort som de gjorde när de undersöktes var för sig.

Ytterligare experiment visade att nedbrytningshastigheten ökar med ökande pH i bärmosen och att högre halt av askorbinsyra leder till ökad nedbrytning. Citronsyra hade ingen effekt åt något håll på antocyaninhalten. Vidare visade sig glukos ha viss nedbrytande effekt vid låga halter (4% w/w) men en skyddande effekt vid 50% w/w.

I färgförsöken så minskade mycket riktigt färgintensiteten med minskad antocyaninhalt i samma batch. När två olika batcher av svarta vinbär jämfördes visade det sig dock att ingen allmän korrelation kunde fastställas.

I de reologiska studierna konstaterades att det fanns skillnader i konsistens mellan de olika batcherna men att ingen korrelation fanns till antocyaninhalten.

Table of Contents

| | |
|--|----|
| Introduction | 6 |
| Aim and purpose | 6 |
| Theoretical Background | 7 |
| Blackcurrant | 7 |
| Bilberry..... | 7 |
| Anthocyanins..... | 8 |
| Spectrophotometry | 11 |
| Colourmetry..... | 12 |
| Rheology | 12 |
| Materials and Methods | 13 |
| Berries | 13 |
| Preparation of berry mash | 13 |
| Thermal treatments..... | 14 |
| Extraction of anthocyanins..... | 15 |
| Measurement of anthocyanin content in extracts | 15 |
| Calculation of anthocyanin content in the berries | 15 |
| Effects of pH-alterations, glucose and organic acid addition..... | 16 |
| Effects of variations in oxygen supply | 16 |
| Colour Measurements..... | 16 |
| Rheology | 17 |
| Data analysis..... | 17 |
| Results and Discussion..... | 18 |
| Effects of heat treatments on anthocyanin content in blackcurrant mash | 18 |
| Effects of glucose and organic acid addition..... | 24 |
| Effects of variations in oxygen supply and separation of skin and pulp..... | 26 |
| Effects of pH alterations..... | 27 |
| Bilberries and Blackcurrant/Bilberry mixes..... | 28 |
| Colour measurements | 29 |
| Rheological measurements..... | 34 |
| Conclusions and future work..... | 37 |
| Acknowledgements | 37 |
| References | 38 |
| Appendix | 41 |
| Appendix 1 | 41 |
| Appendix 2 | 42 |

Introduction

Food and health are currently hot topics in peoples' everyday life. A quick glance at the websites of some of the major newspapers or a visit to the magazine rack of a nearby food store quickly confirms this. The current trend also calls for more old-fashioned products which are perceived as a healthy alternative to the processed food from the industry. Berries and fruits are among those food stuffs which are getting more and more attention for their claimed health benefits and associations to a healthy life style in general.

Simultaneously there has been a lot of research performed over the last decade to verify these claims, beyond already proven health benefits such as the prevention of scurvy, to identify compounds responsible for the observed benefits and their physiological effects on the human body. Prevention of Alzheimer's disease, lower prevalence of cardiovascular diseases and reduction of tumour growth are some of the suggested health benefits that have been described (Bell & Gochenaur 2006, Grace *et al.* 2009, Ping-Hsiao *et al.* 2009).

Among the flavonoids the subgroup anthocyanins have received increasing attention in some of the reported studies. They are found in fruits and berries already regularly consumed by humans such as strawberries, bilberries and red and blue grapes, where they are responsible for most of the (non-green) colour present. From a dietary point of view this means that it is possible to increase the intake of anthocyanins without interfering with peoples' everyday eating habits. But while there have been numerous studies made on model systems on how anthocyanins is affected by different factors such as pH and heating, few studies have been made on the berries or processed products which presents a much more complex system (Rubinskiene *et al.* 2005, Castañeda-Ovando *et al.* 2009, Patras *et al.* 2009).

Aim and purpose

The aim of this master thesis was to investigate how the anthocyanin content in blackcurrants is affected by different chemical and physical factors as well as common ingredients in blackcurrant products. Investigations of how some of these factors influence the colour and texture of the berries have also been made and the results have been compared to the anthocyanin contents of the berries in search for correlations.

Theoretical Background

Blackcurrant

Blackcurrant (*Ribes nigrum*) is a bush that can grow up to 1.5 meters tall. It has 5-10cm long green leaves and the fruit is a very dark blue and purple berry, hence the name blackcurrant. It is native to northern Europe and northern and central Asia and is at present commonly cultivated in cold climates without difficulties. In the wild it prefers to grow along beaches and in moist forests or groves. It is believed to first have been cultivated in the region around the Baltic Sea in the late 16th century and have since become one of the most widespread berries in the region. 450-600ha (15-20%) of the area used for cultivation of berries in Sweden each year is used for blackcurrants, while strawberries as a comparison are grown on up to 75% of the area (GRO 2009). The leaves can be used to make herbal tea or used in pickled cucumber but it is the berries that are the main reason for growing the bushes. The berries are rich in pro-vitamin A and especially vitamin C. Blackcurrant also has high levels of other bioactive compounds like anthocyanins which are discussed in detail in a separate section. (Encyclopaedia Britannica 2009, Nationalencyklopedin 2009)



Figure 1 A blackcurrant twig with leaves and berries

Blackcurrants grown in Sweden are mainly used for jelly, juice and jam. The high natural pectin content together with a low pH in the berries helps explain why it readily gelatinise when high amounts of sugar is added in the jelly production. The rather stringent taste is stated to be the main reason for its near total absence in traditional fruit and berry flavoured products like yoghurt and ice cream. In other countries, like e.g. United Kingdom, it is also used as a pie ingredient, canned fruit or in alcoholic drinks (Hollands *et al.* 2008).

Bilberry

The bilberry bush (*Vaccinium myrtillus*) is a small bush that grows in Europe and Asia. It grows on acidic soil and thrives in the shades in the vast forests of northern Europe. The bush is usually not higher than 40cm and the berries are about 1cm in diameter. The plant is not grown commercially since the berries are fragile and need to be handled with care. In Sweden the plant is estimated to cover 15-20% of the land which helps explaining why the blue berries are nevertheless common in jams, pie fillings and drinks. (Nationalencyklopedin 2010)

Anthocyanins

Chemistry

Anthocyanins are a group of compounds that belongs to the flavonoids which means that they are based on the flavan nucleus. Anthocyanins consist of an anthocyanidin group (fig 2) linked to one or more glycosides through an oxygen atom. More than 20 different anthocyanidins exist but the most common ones are pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidinin (Castañeda-Ovando *et al.* 2009). All of them share the same basic 6C-3C-6C structure shown in figure 2 and the difference is in the substituents on the carbons $R^{3'}$ - $R^{5'}$, R^3 and R^5 - R^7 . These are normally either hydrogen or hydroxyl groups but can also be methyl groups linked through an oxygen atom.

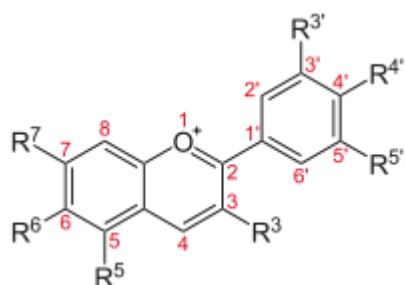


Figure 2 Basic structure of anthocyanidin

The anthocyanidins bind to different glycosides through the oxygen atom normally on the R^3 or the R^5 but the R^7 carbon are also utilised in some cases. Di-glycosides usually bind on the R^3 and R^5 carbons. A large number of sugars can be attached to the anthocyanidins. Commonly occurring are glucose and rutinose but galactose, arabinose and sophorose are also often found. Since a number of modifications on the sugars can occur such as esterification of the sugars with coumaric or caffeic acid but also acetylations of the sugars, the number of anthocyanins that so far are known are in the region of 600. (Coultate, 2002, Yue & Xu, 2008)

Formation

Anthocyanins are formed from phenylalanine, produced through the shikimate pathway, and malonyl-CoA formed from acetyl-CoA. These two are coupled together enzymatically to form a chalcone which continues to be transformed and isomerised enzymatically in 7 steps to form the resulting anthocyanin with the last step being the addition of the sugar substituent on the anthocyanidin molecule. This pathway includes the formation of the flavanone molecule as the precursor for the different anthocyanidins and most of the intermediates are used for production of other metabolites as well, all of this enzymatically regulated. (Winefield 2009)

Occurrence and function in nature

The anthocyanins are produced in nature by a large number of different plants. These range from trees and flowers to carnivorous plants, vegetables and berry carrying bushes. The anthocyanins have a number of specific functions in the plants. One important purpose is to give blue, violet and red colours to the plant as it does in bilberries, strawberries and grapes for instance. The colours are used to attract pollinators to flowers, to signal that the fruits have

ripened and are ready to eat to spread the seeds or as a defence mechanism against insects to name a few depending on intensity and pattern of the colours (Hamilton & Brown 2001, Winefield 2009).

Anthocyanins have been shown to protect green leaves that are exposed to bright light and low temperatures, such as northern Swedish berries, by preventing photoinhibition of the chlorophyll without interfering with the growth (Pietrini *et al.* 2002). They have also been suggested to transport monosaccharides and act as osmotic regulators during drought and low temperatures which could help explain their presence in roots and other tissues not directly exposed to light (Chalker-Scott 1999).

Another important function of the anthocyanins is to act as free radical scavengers. Free radicals, or Reactive Oxidative Species (ROS) as they nowadays are called to include nitrogen containing forms, are produced naturally in chloroplasts in plants during photosynthesis and mitochondria during respiration and may cause damage to cell membranes or the DNA if left uncontrolled. The anthocyanins are able to take care of these in a wide number of ways thanks to their ability to both donate and accept electrons, to stabilise and delocalise unpaired electrons and by chelating metal ions (Winefield 2009)

The colour of the anthocyanins in nature depends on a number of different factors. Differences in pH, co-pigmentation and interactions with metal ions are the factors that mainly determine the colour of the plants. At pH levels around 1 the free flavylium cation (figure 1) is red while at higher pH levels the colour will change to purple and blue colours during transition to the quinoidal base and the subsequent ionised form of it. Higher pH will also lead to the formation of the colourless carbinol pseudo-base and the subsequent chalcone molecule which have a pale yellow colour. All forms except the blue ionised quinoidal base exist in equilibrium, with the flavylium cation as an intermediate, between pH 3-6. This is the normal pH in berry vacuoles so this alone cannot explain the colour of for instance dark blue berries (Coultate 2002). At this pH the co-pigmentation becomes important. The co-pigmentation occurs when compounds interact with the anthocyanin molecule and stabilise the flavylium cation and quinoidal base forms and thereby preventing the colourless carbinols from forming and thus increasing both the red and violet/blue colours of a given concentration of anthocyanins (Cooper-Driver 2001). Examples of such compounds can be other anthocyanins, other flavonols, organic acids, glycosides and nucleotides (Castañeda-Ovando *et al.* 2009). A special case of co-pigmentation is the metal ion complexation which many of the blue or bright blue colours in nature occur from. In these cases a high pH is not needed but it can be in the range of 4-6 (Yoshida *et al.* 2006).

Degradation

Anthocyanins have been shown to be degraded primarily by processing or storage at elevated temperatures (Rubienskene *et al.* 2005, Patras *et al.* 2009) but also at room temperature during storage though at a slower rate (Hager *et al.* 2008). Harbourn *et al.* (2008) showed that the degradation of anthocyanin in blackcurrant juice can be calculated according to the formula:

$$N_{t,T} = N_0 e^{-kt} \text{ (Equation 1)}$$

$N_{t,T}$ is the amount of anthocyanin after the time t at temperature T ,

N_0 is the initial amount anthocyanin k is the degradation constant dependent at the temperature T and t is the time the anthocyanin was held at the temperature T .

This allows the degradation constant to be expressed as:

$$k = -\ln\left(\frac{N_{t,T}}{N_0}\right) * t^{-1} \text{ (Equation 2).}$$

The mechanism for this degradation is not fully comprehended but it has been suggested that it is caused by hydrolysis of the sugar-aglycon bond, opening of the purylum ring or formation of a chalcone. At lower pH other bonds become vulnerable to hydrolysis as well e.g. the bond in disaccharides in the sugar moiety (Patras *et al.* 2009). Kader *et al.* (2001) have shown in a series of experiments that polyphenol oxidase degrades anthocyanins as well by oxidising chlorogenic acid to chlorogenoquinone which reacts with the anthocyanins and form brown condensation products. They also showed that other *o*-quinones can have the same effect but on different anthocyanins so the combination of organic acid and anthocyanin is important. Another important finding was that polyphenol oxidase could not oxidise the anthocyanins without the presence of an organic acid nor in oxygen free environments so all these conditions must be met for the reaction to take place Kader *et al.* (2001). Ascorbic acid have also been shown to degrade anthocyanins at room temperature by a condensation reaction which lowers the content of ascorbic acid and anthocyanin at a 1:1 ratio (De Rosso & Mercadante 2007)

Anthocyanins in blackcurrant and bilberries

Each plant consists of several different anthocyanins but the composition is to some extent specific for each species. In blackcurrants Cyanidin-3-glucoside, cyanidin-3-rutinoside, delphinidin-3-glucoside and delphinidin-3-rutinoside constitute the main part of the anthocyanin content whereas in strawberries and raspberries pelargonidins are more common (Coulton 2002, Rubiensekne *et al.* 2005). In bilberries 15 different anthocyanins have been found and only cyanidin-3-glucoside and delphinidin-3-glucoside are found in both blackcurrants and bilberries (Lätti *et al.* 2008).

The anthocyanins are situated in the seeds, vacuoles in the pulp and the skin but it almost absent in the pulp itself. Their function in blackcurrant and bilberries does not differentiate from the function described above in plants in general (Lu & Foo, 2003, Coulton 2002).

Benefits of anthocyanins for humans

Anthocyanins have been shown to have a number of potential health benefits for humans. Studies have shown that they can help to prevent tumor growth when induced by chemicals, reduce growth and spreading of tumor that have been injected in mice (Ding *et al.* 2006). Further studies have shown that they can help preventing cardiovascular diseases like atherosclerosis (Bell & Gochenaur 2006). They have also been proven to have anti-diabetic properties (Grace *et al.* 2009) and protect against Alzheimer's disease (Ping-Hsiao *et al.* 2009). A problem with this type of studies is that they normally include only one or a few compounds that are being tested simultaneously while berries contain several bioactive compounds. This has the consequence that, even if the results from a single compound study indicates that the compound of interest causes a certain effect, the effect might not be visible if the whole berry or product would be studied. The opposite effect, where several bioactive compounds contribute to a synergistic health beneficial effect, might erroneously be attributed to a single compound (Winefield 2009).

Another issue with anthocyanins is that they have been shown to have low bioavailability. Studies of the bioavailability of anthocyanins have indicated that the uptake in the small intestine may be as low as 1% (Manach *et al.* 2005). However, further studies are needed to investigate if the bioavailability can increase during processing and also during subsequent digestion. Coupled with the degradation during the processing and the storage it might cause a virtually non-existent anthocyanin uptake from a product in which the raw materials has high anthocyanin content. The product would hence wrongfully be marketed as healthy. Further

studies how this affect the potential health benefits from the anthocyanins would thus be of great interest.

Spectrophotometry

Spectrophotometry is the name of techniques used to measure concentrations of different compounds in a solution by using light. Visible light, or what people normally refers to as light, is only a small part of the electromagnetic spectrum. The electromagnetic spectrum ranges from high frequency/short wavelength γ -rays to low frequency/long wavelength radio waves. Molecules can absorb the different wavelengths of radiation and are affected in different ways depending on the energy of the molecule which is related to the frequency according to the equation:

$$E=h\nu \text{ (Equation 3)}$$

E is the energy of the radiation

h is Planck's constant

ν is the frequency

If the energy is high enough the molecular bonds will break and ions might form, like in the case with γ -rays. In the other end of the spectrum are the effects of microwave and infrared radiation which causes movement in the form of rotation and vibration. Since the movement of the molecules is what we call heat, this explains briefly how microwave ovens and infrared heater can be used for heating. When the radiation is in the middle of the scale the molecules will absorb it by exciting electrons and reemit the light by returning to the ground state. Both the absorption and the emission of energy can be measured with relative simple and small equipment and that is what is used in spectrophotometry. (Harris 2007)

UV-Visible absorption spectrometer

The normally used unit to describe the absorption is absorbance. It is calculated by measuring the irradiance (P), energy per second and unit area, sent out by the light source and the compare it to the amount detected by the detector. By comparing these two numbers and using the formula:

$$A = \log\left(\frac{P}{P_0}\right) \text{ (Equation 4)}$$

A is the absorbance,

P is the irradiance at the detector

P_0 is the irradiance at the source

the amount absorbed by the sample can be calculated. This value is of no use by itself though since the value only is relative to itself. To use it a standard curve with known concentrations of the compound of interest need to be established. A dilution series is made and the absorbance is measured and plotted against the concentrations. The value of the inclination of the line can be used as a correlation factor in subsequent calculations. Before measuring the absorbance of the samples of interest the spectrophotometer is set to zero with a sample containing only dilution media, a so called blank. This allows the machine to only measure the contribution from the compound of interest without interference from the dilution media (Harris 2007).

Colourmetry

Colour is one of the main quality indicators when a consumer is assessing a food product. Normally other aspects of the quality are not even considered if the colour is perceived as wrong, e.g. a banana or a pear that is purple would most likely never leave the store (Francis 1995).

To be able to scientifically measure colours and be able to compare different food stuffs or treatment effects on the colour a number of systems have been developed. The first was developed by Commission International de l'Eclairage (CIE) in the early 1930s and consisted of three theoretical colours which together can compose every colour the human eye can see (and some more). This has later been replaced with the Hunter and Munsell systems which use 3D projections to describe the colours on a 2dimensional slab and add lightness on the third axis. In this project the Hunter system will be used. It is using values of a and b to describe the colour in a 2 dimensional plane where a is red, $-a$ is green, b is yellow and $-b$ is blue. By combining these values with an L (light) value ranging from 0 (black) to 100 (white) all different colours can be described (Coultate 2002). This scale is illustrated in figure 3

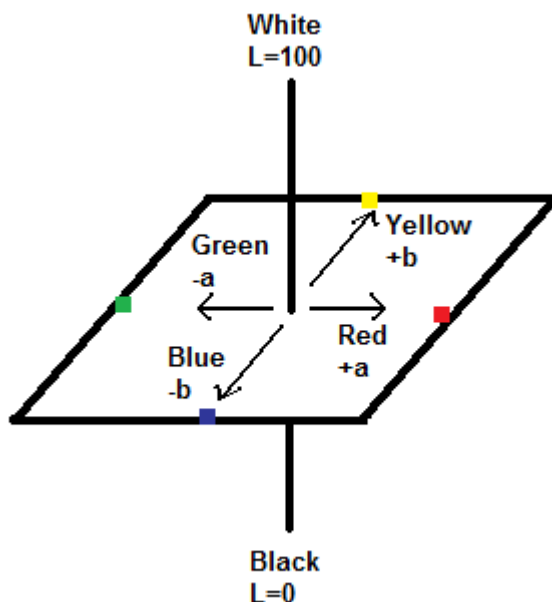


Figure 3 Illustration of Hunter Lab scale

Rheology

Texture is another important aspect of how the customer perceives the foodstuff. Even though the customer may not always be able to tell before purchasing the product if the texture is what he or she expects, it will most certainly help to determine if the product is bought again. It will also affect how the product can be stored and served, e.g. standing by itself on a plate or if it needs to be poured into a glass. In berry products this difference can be seen quite easily by comparing the two common products blackcurrant squash and blackcurrant jelly. Both contains roughly the same amount of blackcurrant juice and while the jelly contains up to 50% more sugar than the squash it is the boiling after the addition of sugar in conjugation with the cooling that causes the pectin in the blackcurrants to gelatinise.

To measure these properties several methods and types of equipment exist. A simple way to compare the relative viscosity of two samples is to use a Bostwick consistometer. It consists of a chamber where the sample of interest is placed, a hatch that allows the sample to be released with high temporal precision when required and a graduated inclined plane on which the sample flows after release. The further the sample flows on the inclined plane in a predetermined amount of time the less viscous it is. This distance is compared to the distance of a reference samples. The value itself is normally not considered scientific enough for usage in research and scientific publications but it is as previously stated useful for comparing different samples to detect differences useful in quality control in the industry.

A more accurate way to measure rheological properties is to use a rheometer. Modern rheometers allow accurate and precise measurements of most rheological properties such as viscosity or shear modulus. The main types of measuring systems used are the rotational cylinder and the cone and plate. There are of course different modifications of both these type of measuring systems and the type used is decided upon depending on the nature of the sample and what information that is expected (Mezger 2006)

Materials and Methods

Berries

Blackcurrants harvested in Västerbotten during September 2009 were provided by Kamrose & Sons HB, Nordmaling. They will be referred to as Batch 1 throughout the thesis. The second batch of blackcurrants were harvested in the Jönköping area in 2009 and purchased from Olle Svensson AB. They will be referred to as Batch 2 throughout the thesis. Both batches were delivered and stored frozen in a cold chamber at -20°C during the project. The bilberries used during the project were picked in northern Sweden in 2008 and provided by Polarica AB, Haparanda. The bilberries were stored in the same cold chamber as the blackcurrants.

Preparation of berry mash

The berries were brought up from the freezer on the morning the same day they were going to be used. They were thawed by filling the beaker they were stored in with lukewarm water and immediately pour it out again 3 times in quick succession and eventually filled with lukewarm water and left standing in room temperature for 5 minutes¹. The beaker was emptied with water and the berries were crushed and shredded with a Bosch hand blender (280W) until a homogenous mash was obtained which held a temperature of 2-5°C. The mash was stored in a beaker at 8°C with the opening covered with parafilm during the course of the day and samples were taken directly from the beaker when needed. Samples from one batch at irregular intervals during 31 hours indicated that the time of samples retrieval did not affect the anthocyanin content since the first and last samples had the highest values and all values were within the standard deviation. This method of thawing and handling was chosen to avoid degradation by heating the berries before treatment, to avoid potential changes during slow unfreezing in a refrigerator overnight or during the weekend and thereby improve the reproducibility and speed up the experimental speed by providing fresh samples when needed. The berries were only used for proper experiments the same day as they were thawed and mashed.

¹ This method of thawing might not be optimal but daily measurements during the first 2 weeks of work showed that the difference between 2 different thawings were of the same magnitude as 2 samples from the same thawing. Hence the decision to continue to use the procedure and focus on more pressing issues.

Throughout these thesis the terms mash, purée and pomace will be used frequently to describe the treated berries. Mash will be used to describe whole berries that have been thawed and destroyed by a hand blender. Purée will be used to describe mash that have been sieved through a 0.5mm or 1mm metal sieve and thus had most of the seeds and larger skin particles removed which will be described as the pomace. Mixture will be used to describe berry mash combined with either another kind of berry mash such as bilberry/blackcurrant combinations or a mash with glucose or any other chemical added to it.

Thermal treatments



Figure 4 Picture of the equipment used for heating. The headspace vials were placed in the 4 small holes while the big hole allowed the stirrer to rotate freely while staying in the centre. The white rod is the thermometer of the bath. The glycerol has a brownish colour due to berry residue.

For the heating experiments, 12ml or 20ml headspace vials (Chromacol, United Kingdom) were filled with blackcurrant mash. A small magnetic stirrer was placed at the bottom of the vials before the mash was added in all samples to improve the heat transfer (figure 16). The vials were sealed with caps using a hand crimper.

When the samples had been prepared and sealed the vials were put into a glycerol bath of the desired temperature (Figure 4). Glycerol was chosen as heating medium due to its high boiling point, low toxicity and availability in the lab and allowed all temperatures of interest to be tested in the same setup. Glycerol level in the pot was maintained at a level covering all of the vials except the caps. When switching from large to small vials excessive glycerol was removed. The vials were kept in place by a plastic frame and a magnetic stirrer was used to

stir the glycerol and consequently ensure proper heat transfer and a uniform temperature in the bath (figure 4).

During initial trials, the time to reach the same temperature in the vials as in the surrounding glycerol was concluded to be close to 5 minutes (see appendix 1) regardless of temperature. This data was obtained by placing a thermometer in one of the four samples used during the first weeks of the project. The time used in the graphs and calculations is the holding time at the set temperature

After the samples had been heated at the desired temperature/holding time settings, the vials were transferred into an ice bath for cooling. The temperature of the ice bath was 1-3°C which ensured a high temperature gradient throughout the cooling and thereby a rapid cooling. The chilling to room temperature never lasted longer than 2 minutes and melted ice was replenished continuously.

Following the subsequent removal of the caps, samples of the treated berry mash could be retrieved and used for anthocyanin analysis or further experiments.

Extraction of anthocyanins

5g of each treated sample were put into an E-flask together with 45ml methanol for extraction and 0.5ml of 37% hydrochloric acid to obtain a pH of 1-2 and keep the anthocyanins stable. The flasks were heavily shaken to obtain proper mixing. The openings of the E-flasks were covered with parafilm and the whole flasks were wrapped in aluminium foil. The sample was stored in a refrigerator at 8°C over night for extraction and to prevent further degradation. Duplicate samples were taken in all cases.

Measurement of anthocyanin content in extracts

The extracts was retrieved from fridge the next day and filtered through a filter paper grade 00R (Munktell Filter AB Sweden) using a funnel and collected in another E-flask. Mechanical pressure was applied on the berry residue to prevent it from keeping too much of the methanol and juice in the filter cake. 5ml of 60% methanol in water (v/v) containing 1% HCl (v/v) was poured into beakers and a set amount of filtrate was added. The amount of filtrate used in each dilution was decided upon by trial and error since the subsequent measurement of absorbance was supposed to be around 0.5-0.6. 2.5-3 ml of the dilution was poured into a plastic cuvette and the absorbance at 530nm was measured in a spectrophotometer (Ultrospec 100, Pharmacia Biotech, Sweden). The methanol solution used for dilution of the filtrate was used as reference blank. All measurements were made in triplicate which totalled 6 measurements for each treatment.

Calculation of anthocyanin content in the berries

A correlation factor between the amount of anthocyanin in a sample and absorbance was obtained by preparation of a standard curve using a solution containing cyanidine-3-glucoside bought from Polyphenols, Norway and methanol. The correlation factor was found to be 0.0416 (see appendix 2)

The anthocyanin content left in the berry mash before and after treatments was calculated using equation 5:

$$\text{mg anthocyanin/g berry (wet weight)} = \frac{ABS * V_{\text{sample}}(ml) * (V_{\text{dilution}}(ml) + V_{\text{extract}}(ml))}{c * V_{\text{extract}}(ml) * 1000 * m_{\text{berry}}(g)} \quad (\text{Eq. 5})$$

ABS is the measured absorbance

c is the correlation factor from the standard curve

V_{sample} is the amount of methanol used for extraction

V_{dilution} is the amount of 60% methanol mixture used to dilute the extract

V_{extract} is the amount of filtered berry mash/methanol pipetted into the 60% methanol for the measurements,

the factor 1000 transforms the result to mg/g instead of $\mu\text{g/g}$ which is obtained from the standard curve

m_{berry} is the amount of berry in the extraction beaker.

Effects of pH-alterations, glucose and organic acid addition

Samples with different pH levels were prepared by addition of solid sodium hydroxide to 50g of berry mash. The mixture was stirred until the sodium hydroxide was completely dissolved and pH of the samples was measured in the vials before and after heating. pH was readjusted to between 1-2 for the anthocyanin extraction and measurement.

Ascorbic acid, citric acid and glucose were added in solid form to the berry mash as described above and the mixtures were stirred until the samples were deemed to be homogenous.

A time-temperature setting of 60minutes holding time at 100°C were chosen for these tests and the results was compared to those obtained from heating the otherwise untreated berry mash and the raw reference samples.

Effects of variations in oxygen supply

The effects of air supply were tested by filling 20ml vials with 12g of berry mash with took half of the volume of the vials, i.e. 10ml. By leaving the vials open the effect of free access to oxygen could be investigated. Effects of limited air supply and completely oxygen free environment were tested by sealing of the vials with regular air inside and in the latter case by flushing the vial with nitrogen immediately before sealing.

Similar to the tests described above a time-temperature setting of 60minutes holding time at 100°C were chosen and the results was compared to those obtained from heating the otherwise untreated berry mash and the raw reference samples.

Colour Measurements

A method for evaluating colour changes was developed in the beginning of the experimental work. The residue left in the vials when the 5g needed for anthocyanin extraction had been removed was used for the colour measurements. The residue was placed on a 0.500mm sieve and mechanical pressure was applied by a microspatula to squeeze out purée and juice from the berry mash. This removed the seeds and other large particles, such as pieces of skin, which affected the measurements initially. The purée was collected on a watch glass and 0.5g of the purée was placed in a small container and diluted and mixed with 5ml of distilled water until a

homogenous solution was obtained. The water raised the pH from 2.9-3.0 to 3.3 but no colour difference could be detected due to this increase.

These solutions were pipetted onto a white porcelain spot dish in two separate wells. The wells were covered with standard sized objective slides which sealed the wells by capillary forces and thus creating a confined space of the same volume each time as long as no air was left in the well. The colourmeter (Konica Minolta Colour Reader CR-10, Konica Minolta, Japan) was placed on top of the objective slide and the colour was measured 3 times for each well (the colourmeter was moved and reset between each measurement). The measurements were performed in the same room and place each time to assure that the same amount of background light always was present. The colourmeter was calibrated using the white plate included in the colourmetric equipment.

Rheology

For rheological measurements the berry mash obtained from heating treatments was filtered through a 1mm sieve. The berries were pressed with a spoon until further pressure stopped yielding more juice and purée. The sieved purée was continuously scraped off into a small glass bowl. The purée was placed in a 30mm cup in the Stresstech HR Rheometer (ReoLogica Instruments AB Lund, Sweden). Viscosity measurements were performed with a “bob in cup” CC30 and settings were adapted to the individual samples. The tests performed were of the shear rate ramp (CSR) type. The stress was measured at shear rates between 1-500s⁻¹. The interval was chosen since chewing and swallowing has a shear rate of 10-100s⁻¹ (Mezger 2006) and the extensions of the interval were made to include any effects right outside or in the vicinity of this region. Between 5 and 12 measurements on each sample was performed depending on how good the readings were, with a ratio of 0s to 1s of at least 6:4 and no more than 20% of the 1s could be outside 1.0±0.1 for the results to be considered accurate. Each treatment was repeated twice and the treatments that were evaluated were selected to give a broad but still quick picture of how the consistency could be related to the anthocyanin content.

Data analysis

The data analysis and degradation modelling was performed with Microsoft Excel. The standard deviations presented with the data were calculated by using the standard deviation tool in Excel. Linear regression on the logarithmic values was used to calculate the degradation coefficient, k , at the different temperatures. The Solver tool in Excel was used to calculate the degradation coefficient by minimising the Sum of Squares (SoS) when applicable.

Results and Discussion

Effects of heat treatments on anthocyanin content in blackcurrant mash

The effects of heat treatment at 80°C, 100°C and 120°C on the anthocyanin content was measured during 90 minutes for samples heated at 80°C and 120°C and up to 360 minutes for the 100°C (figure 7). The anthocyanin content was found to decrease in all samples during heat treatment and the most rapid degradation was observed at the higher temperatures (figure 5).

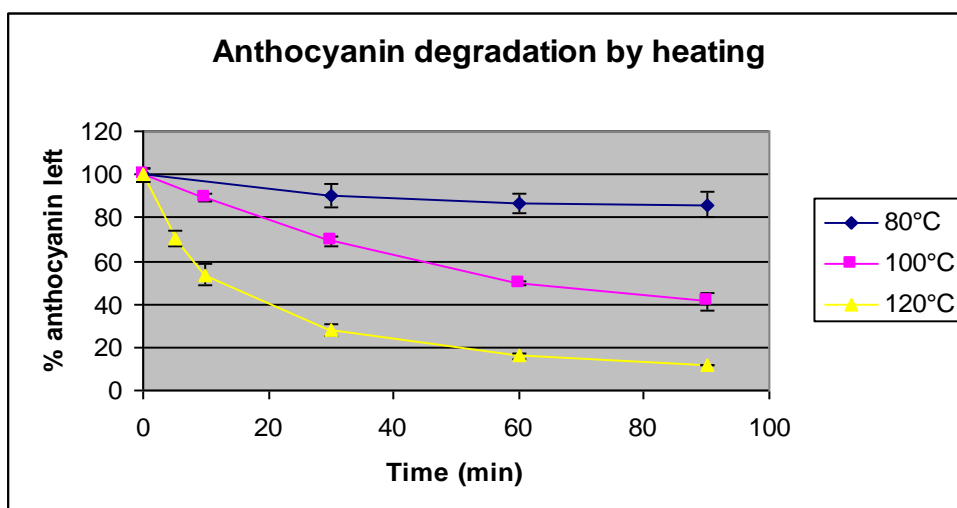


Figure 5 The time-temperature dependence of anthocyanin degradation in blackcurrants

Effects of heating at 80°C

At 80°C the degradation of anthocyanin was slow and less than 15% of the content had disappeared after 90 minutes. No apparent difference in smell or appearance could be detected when the vials were opened after treatment. The experimental degradation curve does not totally correspond with the calculated degradation curve and has a k value of 0.0020 (degradation constant, see Eq 1) and R^2 -value of 0.77. All the data points for the calculated degradation curve were within the standard deviation of the measured data. Compared to the literature value for k , 0.0013, (Harbourne *et al.* 2008) the degradation was faster at all measured or calculated points.

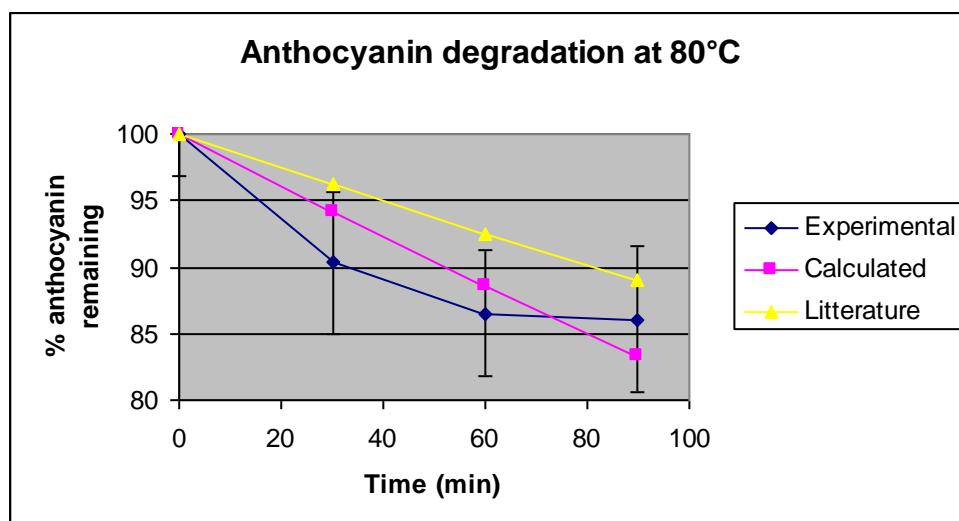


Figure 6 Comparison of the experimental degradation data, the calculated degradation curves, both by linear regression and sum of squares, and the degradation curve predicted by literature data at 80°C. Both the calculated curves are exponential but the scale is too small for it to be noticeable.

Effects of heating at 100°C

At 100°C the anthocyanin was degraded faster than at 80°C but the degradation speed decreased after 2 hours and was lower during the last 4 of the 6 hours investigated. The calculated degradation constant, 0.0063, results in a degradation curve between the one predicted in the literature and the experimental data and has a R^2 of 0.87. If only the first two hours is measured a degradation constant of $k=0.0097$ is obtained which results in a curve which have a fit of 0.97 which is a really good prediction. The half life at 100°C was calculated to be 110 minutes when using the constant obtained at the 6 hour interval but only 71 minutes when using the degradation constant obtained from the first 2 hours. When comparing these results with the degradation constant reported by Harbourne *et al.* (2008) it is obvious that the degradation is much faster in the experimental data and both predicted models compared to the literature value (Harbourne *et al.* 2008).

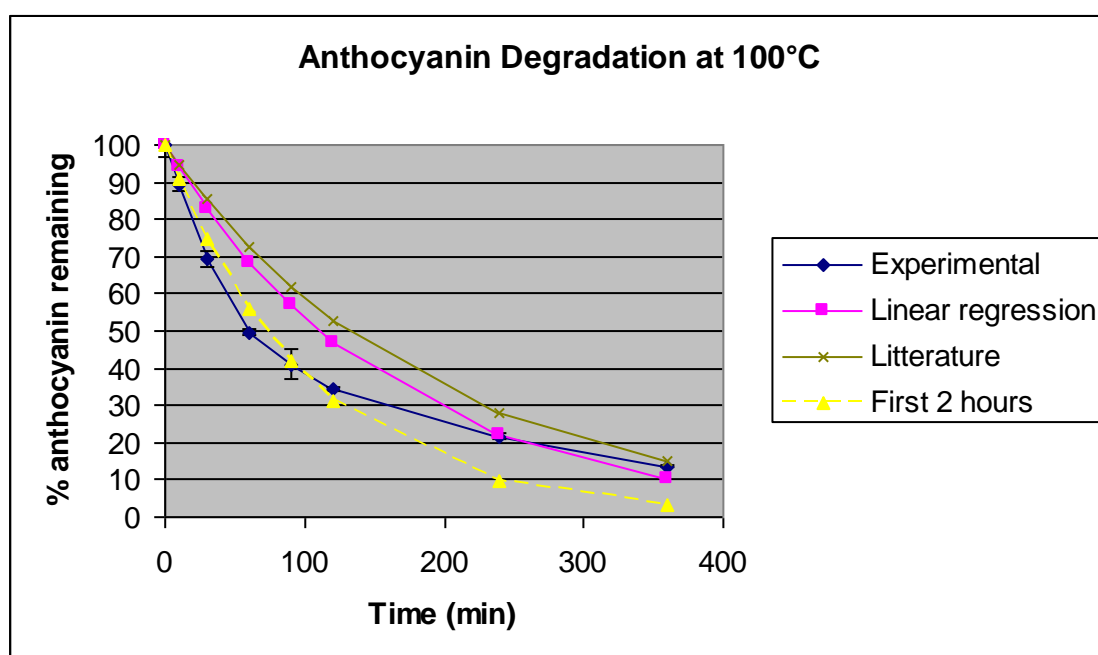


Figure 7 Comparison showing the experimental data compared to 3 different predicted degradation curves at 100°C. The k obtained both by linear regression on the data from the first 2 hours and the Sum of Squares method makes an excellent prediction for the first 2 hours but overestimates the degradation in the end.

Effects of heating at 120°C

At 120°C the degradation starts early and at a high speed. After 5 minutes up to 30% of the anthocyanin content has been degraded and after 10 minutes almost half of the anthocyanin content has disappeared. The samples shifted from a purple/red colour to a brighter red and brown shade and the samples had an unpleasant scent when opened including those with as low holding times as 5 minutes. The measured degradation was much faster during the first hour than the calculated degradation on the whole interval which had a degradation constant of 0.0276 with a R^2 value of 0.83. No literature value to compare with could be found due to differences in heating and cooling techniques. Linear regression on the interval 0-30 minutes led to approximately the same degradation constant as obtained by minimising the sum of squares, $k=0.0427$ $R^2=0.90$ and $k=0.044$ respectively. These predicted that the anthocyanin concentration should be almost 0 after 90 minutes but this could not be observed. There were still detectable amounts of anthocyanins after 6 hours at 120°C, however it was as low as 2.7% of the original content (data not shown). The measured half life was approximately 10 minutes while the predicted by linear regression on the whole interval was as long as 25 minutes.

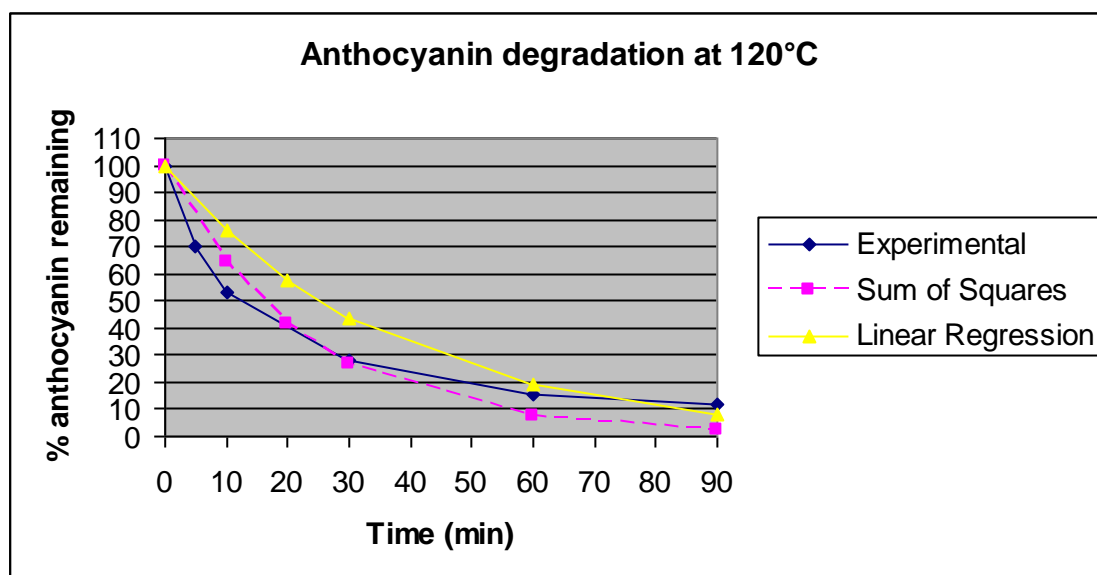


Figure 8 Comparison between the experimental data and the data calculated from linear regression on the whole interval and the first 30 minutes/sum of squares. The calculated degradation underestimates the initial degradation but reaches the same level in the end while the sum of squares model offer a better initial fit.

Comparison of Batch 1 and Batch 2

Two batches of blackcurrants were used for the experimental work since the amount of berries in the first batch was not adequate. Hence it became of great importance to compare the degradation in the two batches to see if the results obtained from the first batch could be compared with the results from the second batch. The degradation of the anthocyanin content in both these batches of blackcurrants was compared at 100°C and 120°C. The first batch had an initial content of 4.8mg anthocyanin/g blackcurrant (wet) and the second batch had a concentration of 3.6mg anthocyanin/g blackcurrant (wet). Although the concentration differences decreased towards the end, especially at 120°C (Figure 9), it was obvious that a different calculation method was needed.

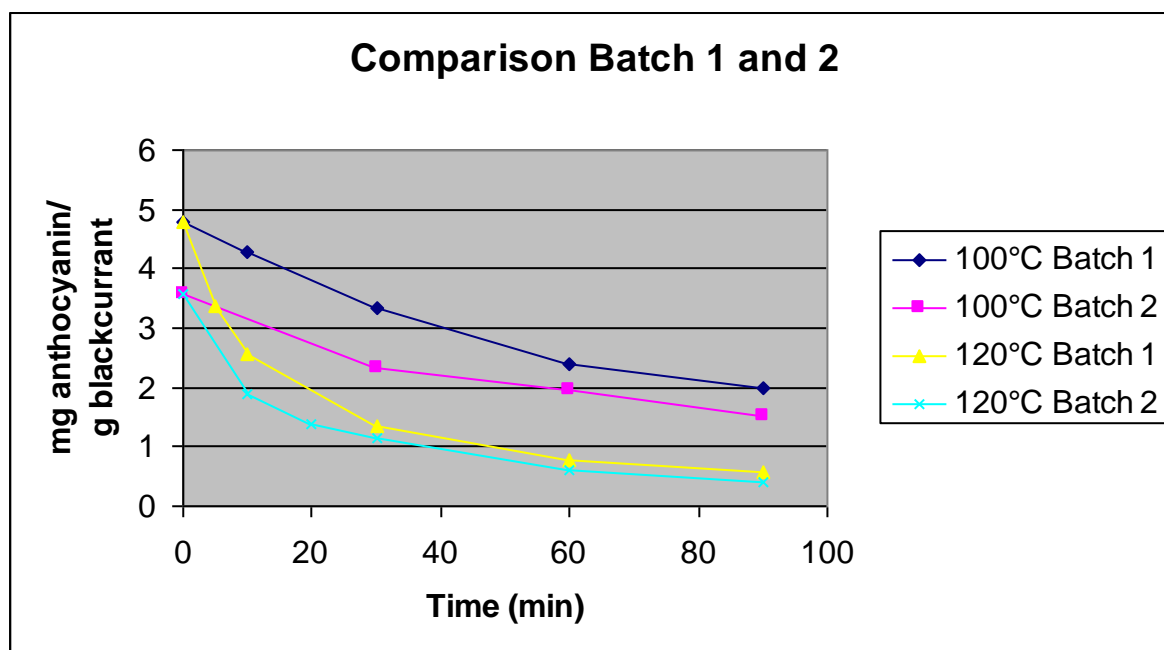


Figure 9 Comparison of anthocyanin concentration and degradation in the two different blackcurrant batches at 100°C and 120°C

The problem was solved by setting the initial amount of anthocyanin in both batches to 100% and compare the amounts remaining in the treated samples to the initial amount in respective blackcurrant batch. When comparing the degradation curves of the two batches with the values recalculated to relative amounts instead of actual amounts, the difference between the batches were virtually nonexistent (Figure 10) and this allowed the data obtained from the first batch to be compared to the second batch. Since all blackcurrants mainly contain four anthocyanins and in the same ratios (Rubinskiene *et al.* (2006)), the shared degradation curves were expected but still needed to be confirmed. Henceforth all the anthocyanin data, unless specifically stated, will be presented as the final amount relative to the initial amount.

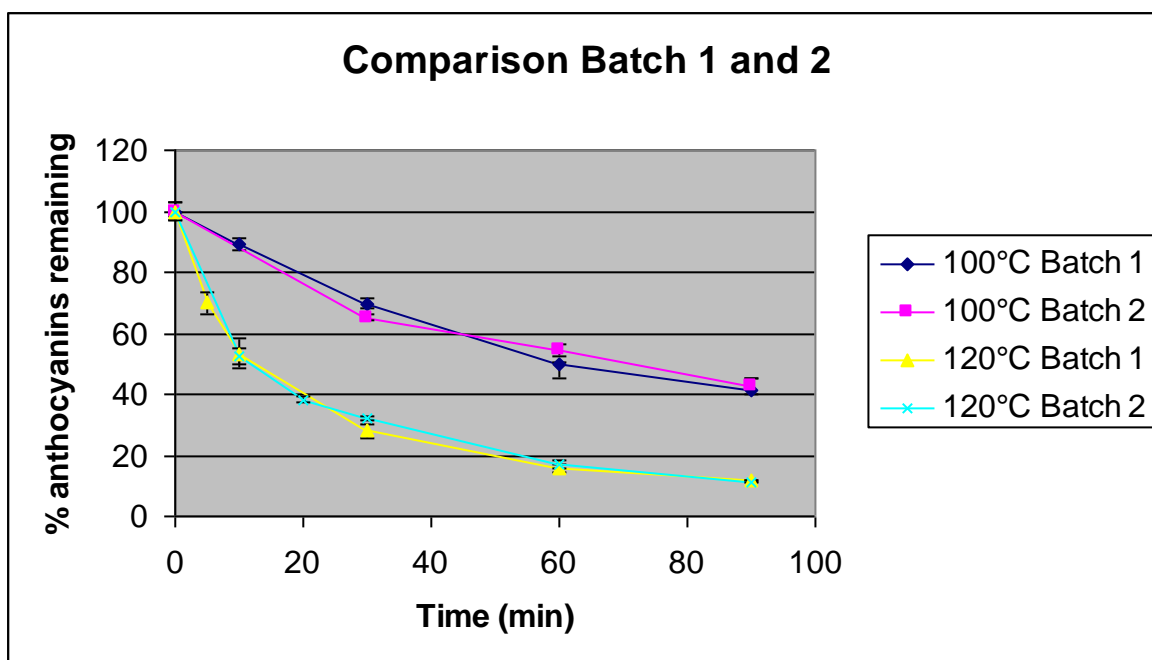


Figure 10 Comparison showing that for each time and temperature, C/C_0 has more or less identical values for both batches of blackcurrant.

Final discussion regarding heat treatment

At the temperatures investigated more thoroughly, 100°C and 120°C, the degradation speed could be calculated with the formula outlined by Harbourne (2008) during the initial 30-60 minutes. The degradation started to diverge at an increasing rate from the model as holding times increased. The degradation became slower and slower and the amount of anthocyanin never reached below 2.5% even at a holding time of 6 hours at 120°C (data not shown). A possible explanation is that the method used to measure the anthocyanin concentration, in which it is assumed that everything that absorbs at 530nm is anthocyanin, might be a simplification of the situation. The amount present in raw blackcurrants are within the range presented in literature (Hollands *et al.* 2008) which infers that the method is accurate enough but it is not established whether some of the degradation products also absorb at 530nm. HPLC experiments on the samples could be performed to investigate whether there is any anthocyanin left, if it is degradation products that absorb the UV-light or if it is a combination of both. Another explanation could be that the blackcurrant matrix slows down the degradation by protecting the anthocyanins in the seeds. Since the extraction took up to 24 hours, and thus allowing more time for the anthocyanins to dissolve into the methanol solution, this could help explaining why they could be detected anyway.

Effects of glucose and organic acid addition

The reason for testing citric acid, ascorbic acid and glucose was that they are natural components of fruits and berries and common ingredients in fruit and berry products. Citric acid is added as an acidifier in industry to most fruit and berry products and ascorbic acid is added to e.g. blackcurrant squash. A quick scan on the internet of homemade recipes verifies that at least one of these acids normally is included in jams and other homemade preserves. The glucose was chosen as a sugar representative due to already being present in the samples in the form of sugar moieties on two of the four anthocyanins and its presence in sucrose in the berries. Since the preparation of berry preserves requires heating or boiling, and in the case of jelly preparation cooling due to the pectin, the berries were heated at 100°C for 60 minutes.

The addition of citric acid (CA), ascorbic acid (AA) and glucose yielded different results. The citric acid addition yielded no noticeable difference, neither at 0.4% (w/w) nor at 4% (w/w), compared to the reference sample (49.7% left compared to 49.8% and 49.4% respectively). The pH in the 0.4% sample showed a minor increase from 2.9 to 3.0 while it decreased to 2.5-2.6 in the 4% (w/w) sample.

The sample with 0.4% (w/w) ascorbic acid did not affect the anthocyanin degradation (50.2% left) but the sample containing 4% (w/w) showed a faster degradation than the other samples and only had 42.1% of the initial content left. The pH varied only slightly between the samples and all was in the range between pH 2.95 -3.05.

The effects of glucose addition varied depending on the amount of glucose added. When 4% (w/w) of the samples consisted of added glucose the amount of anthocyanin decreased to 46.7% of the original content. The samples with 50% (w/w) of glucose added showed an increase in the anthocyanin retention and had 56.1% of the initial content left after one hour at 100°C.

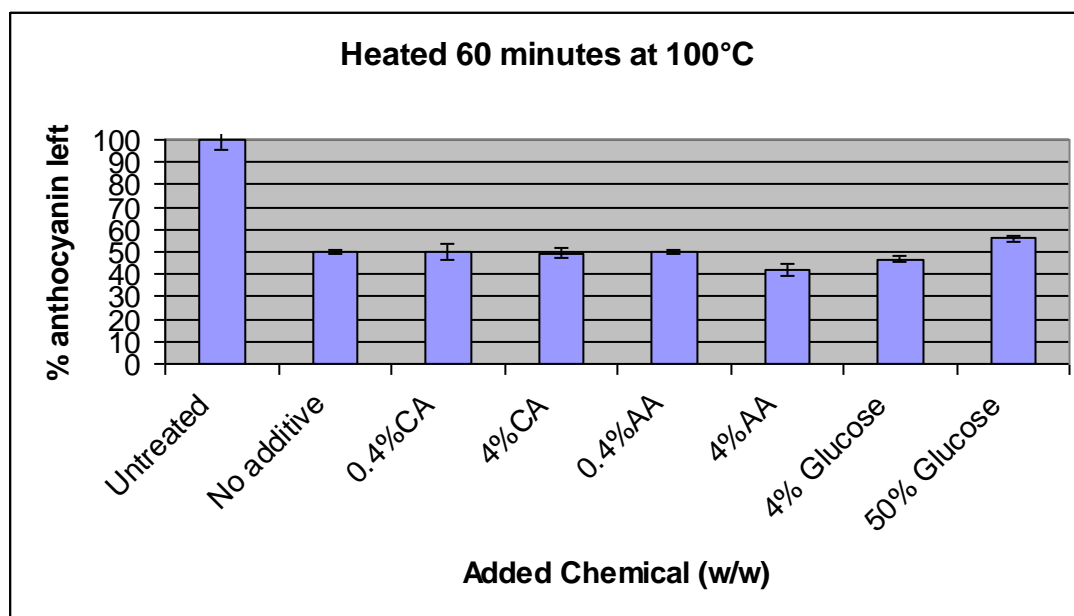


Figure 11 The addition of 3 different common food additives in two different concentrations and their effect on the anthocyanin content compared to samples with berry mash only.

The citric acid caused as stated above no effect neither at amounts present in recipes (0.4% w/w) nor with the tenfold increase. Citric acid is not known to interact with anthocyanins and the decrease of pH as in the case with 4% (w/w) should only act to stabilise the anthocyanins. The fact that no such effect could be seen indicates that at a pH below 3 the anthocyanins were not stabilised to any further extent than what already were the case. Citric acid addition does not affect the nutritional value of the product and can be added early in the processing if required.

The addition of low concentrations (0.4% w/w) of ascorbic acid did not cause any difference. This is the amount used in the homemade recipes and the results indicates that this amount causes no further degradation and it is the temperature and holding time that should be minimised to retain maximal values of anthocyanin. When the added amount was increased tenfold, the degradation speed increased greatly from 50% to almost 60% in one hour at 100°C. According to a study done by De Rosso & Mercadante (2007) a higher concentration of ascorbic acid increases the degradation of anthocyanins in berry extracts in room temperature. The reason that this was not noticeable at the lower concentrations could be explained by the fact that blackcurrants already contains 0.2% w/w ascorbic acid and an increase to 0.6% w/w does not seem to be enough to cause a noticeable difference. The addition of 4g ascorbic acid /100g blackcurrants caused the ascorbic acid concentration to rise to 4.2% w/w and this is evidently enough for a detectable difference. Poei-Langston & Wrolstad (1981) showed that the ascorbic acid and anthocyanin react through a condensation reaction and thereby reduce the content of both at a 1:1 ratio. In this experiment $5.68 \cdot 10^{-3}$ moles of ascorbic acid increased the degradation by $3.36 \cdot 10^{-6}$ moles of anthocyanin (calculated from average molecular weight of 565g/mole according to data from Rubinskiene *et al.* (2006)), i.e. 0.06% of the ascorbic acid might have reacted with anthocyanins. This might seem low but it can be attributed to the fact that it was only allowed to react for a little more than one hour, the berry mixture is a less than ideal system for chemical reactions and that ascorbic acid itself is degraded by heat.

The increased amount of anthocyanin left when 50% w/w glucose was added can most likely be attributed to the lower water activity that the high amount of glucose cause. Lowering the water activity has been shown to decrease the degradation of dried anthocyanins solved in water and this seems to be the case even in the berry mixture (Rubinskiene *et al.* (2006)). The same study also showed that concentrations of sugars below 20% w/w decreased the anthocyanin content which is consistent with the results obtained from the 4% sample.

Effects of variations in oxygen supply and separation of skin and pulp

The effect of different amounts of oxygen and mechanical treatments yielded varying results. The sealed samples with regular air and nitrogen showed minor increases in the anthocyanin content left compared to the reference sample (52.6% and 51.1% respectively compared to 49.6%).

The samples which had its opening unsealed and thereby allowing heat and mass transfer into the surrounding room showed a larger retention of the anthocyanins with 55.2% left. These samples lost up to 8% of their weight which have been included in the calculations. The lack of sealing increased the heat transfer to the surroundings and caused a heat gradient and lower temperature on average in the samples which might have slowed the degradation.

The results from the measurements in which mashed berries were pressed through a 1mm sieve were in agreement with earlier studies describing that most of the anthocyanin is situated in the skin of the berries (Coultaite 2002). The filtered purée only had 36.8% of the anthocyanin content of the untreated mash left after treatment while the pomace had 61.2% of the original content left. If these two concentrations are added the resulting anthocyanin content becomes 48.9% of original content which is only a minor decrease compared to the reference sample (49.6%).

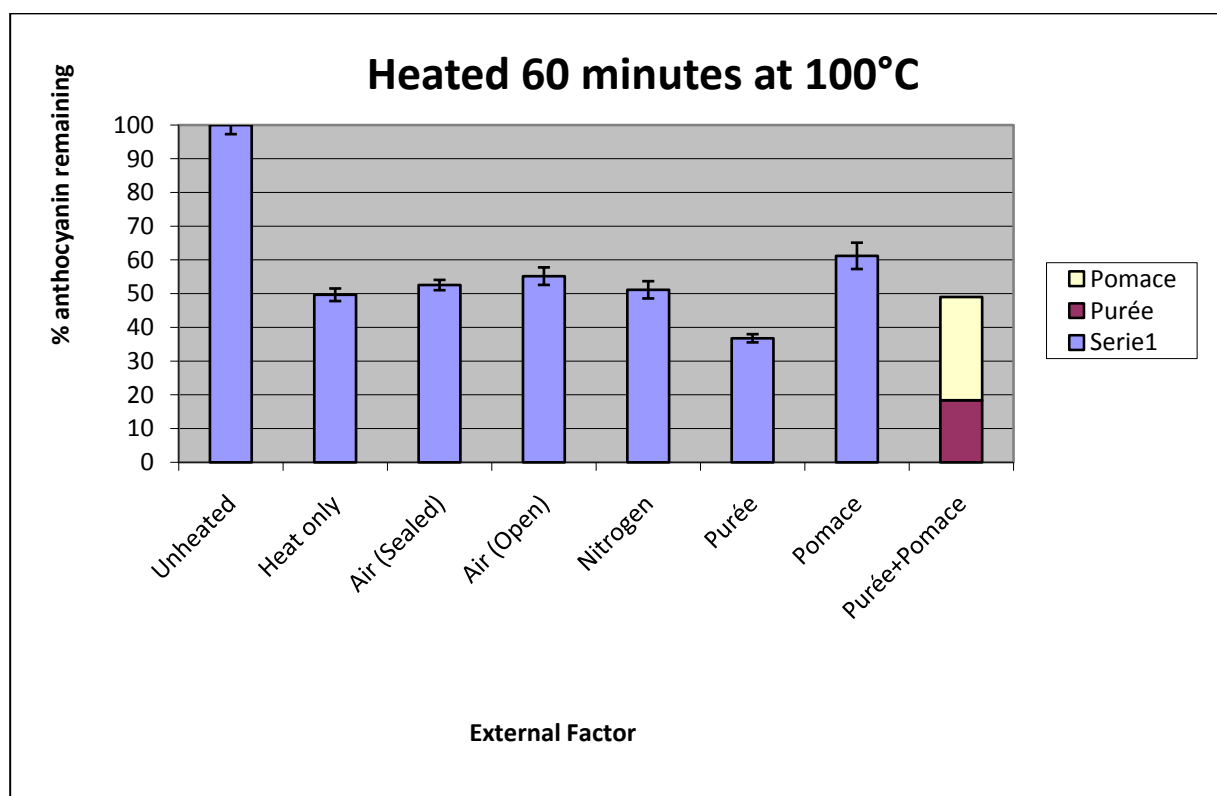


Figure 12

Comparison of external factors affecting the anthocyanin content. The rightmost bar shows that the anthocyanin content in the purée and pomace samples totals to more or less the same amount as in the complete berry mixtures.

Effects of pH alterations

Addition of sodium hydroxide and hydrochloric acid during initial screenings showed that both these compounds lowered the amount of remaining anthocyanins after heat treatment compared to unaltered but heated berry mash. Since most products are heated to some extent during preparation it was investigated if an altered pH during the heating would affect the anthocyanin content irreversibly. The pH was lowered to pH 1-2 at extraction and analysis.

The addition of 1ml 37% HCl lowered the pH to 0.5 and decreased the anthocyanin content left after treatment to 47.2% of the original content. The addition of 4% (w/w) of NaOH increased the pH to 9.7 and decreased the remaining anthocyanin content to 12.2% of the original content. The subsequent studies that these findings led to showed that the anthocyanin content decreased linearly with the rising pH level between pH 3 and pH 8 whereas no further degradation could be seen with the increase to pH 9.7. The colour of the sample changed from red/purple to dark purple, green and brown/black in the sample with the highest pH. Linear regression on the anthocyanin content in the pH 3-8 interval resulted in a function which yielded 8.2% less anthocyanin remaining compared to the initial amount when pH increased by 1. The R^2 -value for this line was 0.988 which is a good prediction. The fact that there were no further decrease of anthocyanins at pH 9.7 might be attributed to coloured degradation products as described in the heating section. That suspicion is reinforced by the earlier results that only showed lower anthocyanin content on two occasions which were the 4 hours and 6 hours treatment at 120°C.

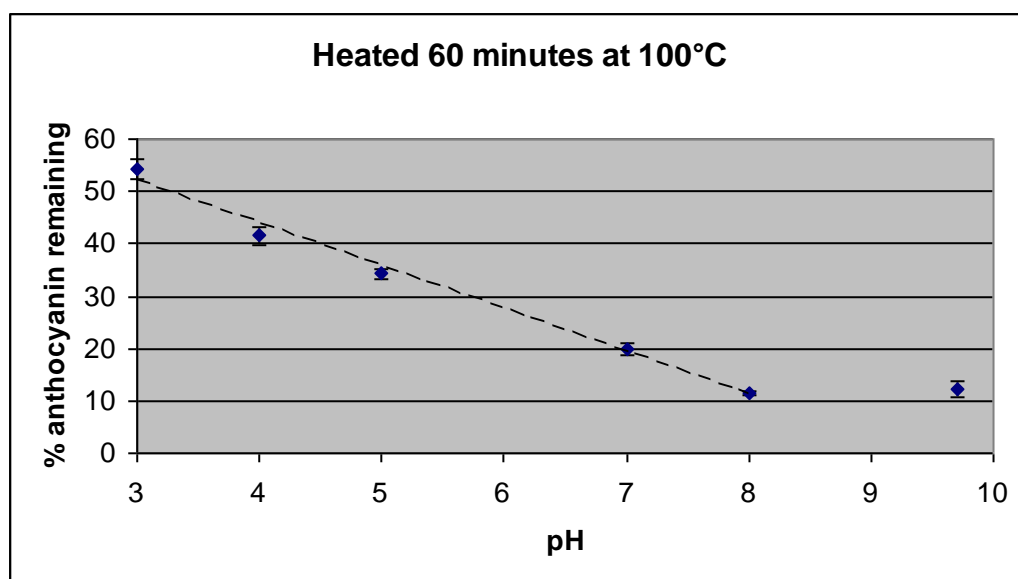


Figure 13 The increased degradation of anthocyanins with increased pH.

It has been established how the anthocyanins form different compounds and lose their colour when the pH increases but this is normally reversible when the pH decreases again (Coultate 2002). Early experiments in the laboratory on the blackcurrant mash, where sodium hydroxide and hydrochloric acid were added in arbitrary amounts, changed the colour from the regular red/purple to almost completely green/black and later back to the red and purple. This phenomenon was not observed in the case of the pH elevated and heat treated samples. When the pH was adjusted to 1-2 for extraction the samples still had different colours and hues compared to the control samples but also compared with each other. The colour differences persisted through the night and were still present at the anthocyanin measurements the next day but the pH remained unchanged at 1-2. These observations support the theory that the

combined pH increase and heating caused irreversible degradation of the anthocyanins in the berry mash. The slightly lower amount of anthocyanin in the sample with only hydrochloric acid can probably be explained by the fact that the pH was not adjusted properly and it remained below the absorption maximum of pH1-2.

Bilberries and Blackcurrant/Bilberry mixes

The heating experiments performed on bilberries yielded similar results as the blackcurrant experiments. Both for 100°C and 120°C an exponential degradation curve was obtained though with different degradation coefficients than for the blackcurrants. They were concluded to be 0.0142 and 0.0396 with R^2 values of 0.87 and 0.85 for the 100°C and 120°C treatments respectively. As in the case with blackcurrants the exponential model on the entire interval underestimates the initial degradation but reaches more or less the same endpoints. As can be seen in figure 17 anthocyanins in bilberries are degraded faster than in blackcurrants.

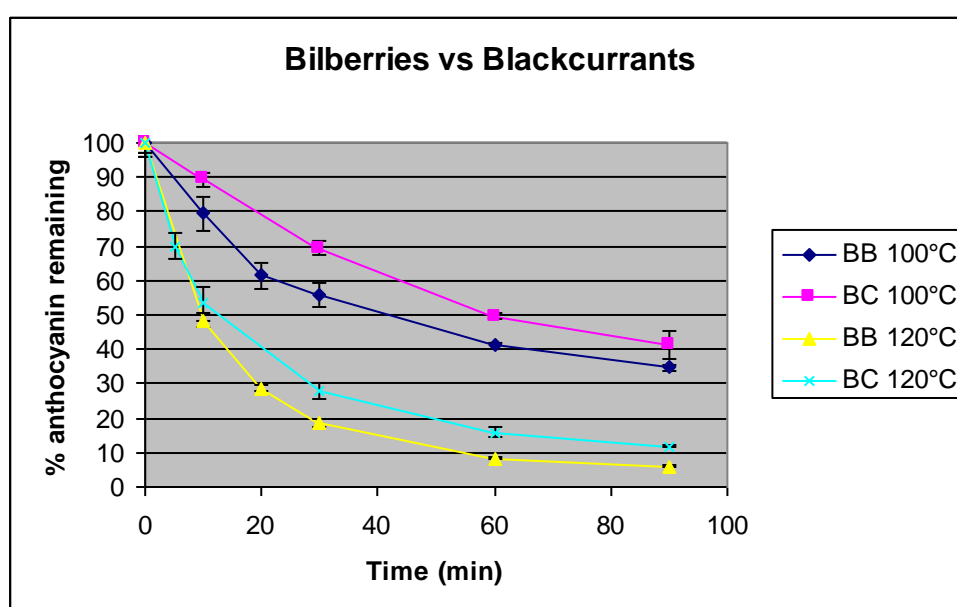


Figure 14 Comparison between the degradation curves for blackcurrant (BC) and bilberries (BB) at 100°C and 120°C.

The reason for the different degradation rates is most likely due to the different anthocyanin composition of bilberries and blackcurrants. As shown by Rubinskiene *et al.* (2005) the four different anthocyanins present in blackcurrants are not equally heat resistant. In bilberries there are 15 different anthocyanins and only cyanidin-3-glucoside and delphinidin-3-glucoside are found in both blackcurrants and bilberries (Lätti *et al.* 2008).

A mixture with 50% bilberries and 50% blackcurrants (w/w) were subject to the same heat treatment at 100°C as the pure blackcurrant and bilberry samples and the anthocyanin content was compared to the values predicted by combining the results from the single berry samples. The results showed that there is most likely no interaction between them and that the total amount of anthocyanin in the mixture is close to identical to the amount predicted from the values obtained by combining the values from the single berry samples. This implies that if the aim is to maximise the anthocyanin content in a mixture there is no difference if the berries are mixed before or after the heat treatment (figure 14)

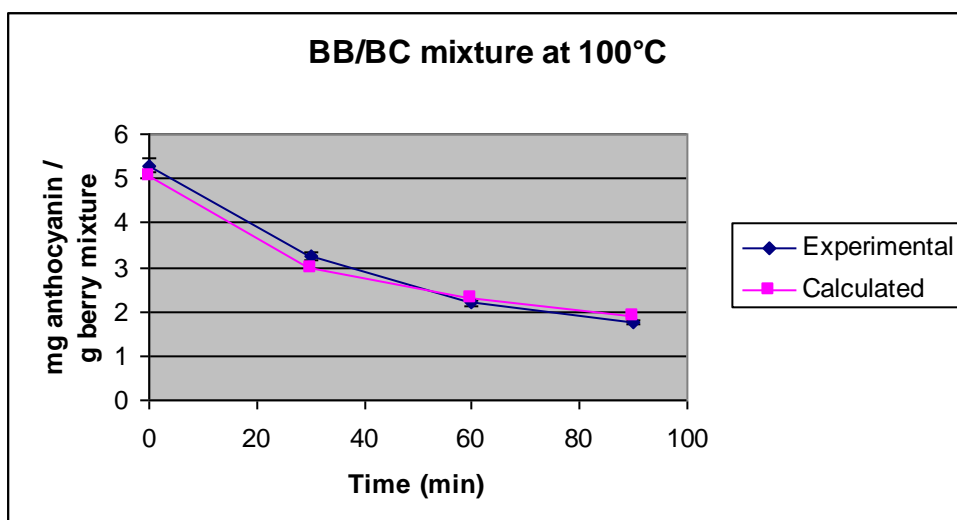


Figure 15

The almost identical graphs indicate that there is no apparent difference in mixing two the berries at a 50/50 (w/w) ratio before or after the heat treatment.

Colour measurements

The colour was concluded to be affected by heating and other treatments, most notably pH changes, quite early during the experiments. The challenge was to perform reliable measurements, not only visual inspections of 2 different samples. This is illustrated in figure 16 which compares raw untreated blackcurrant mash with blackcurrant mash heated 90 minutes at 120°C, i.e. the two extremes in the project regarding anthocyanin content. Even though they are quite easily distinguished all other samples are between these two and are difficult to distinguish from one another, especially if the differences are small. These difficulties were illustrated even better when comparing the extracts used for measuring the anthocyanin content as described earlier. When these extracts were stored in beakers personnel in the lab was asked to tell if they could see any differences in colour between undiluted raw extracts and undiluted extracts with 50% less anthocyanins and most of them failed to spot any (controls were made by asking them to compare the colour of two beakers with the same content which yielded the same results). However, the differences in intensity was quickly noticed though when the extracts were diluted in the 60% methanol solutions for anthocyanin measurements.

The results from the first batch show no apparent loss of colour in the samples heated at 80°C. At 100°C the *a*-values remained virtually unchanged while a gradual decrease of the *b*-values can be seen with the decrease in anthocyanin content. At 120°C the loss of colour is noticeable early on and becomes obvious at the longer treatment times when it is clearly noticeable, even for the human eye (fig 16). The *L*-values remained unchanged for all samples and will henceforward not be included in the discussion since it seemed to be affected more by the amount of particles than the colour of them.



Figure 16 Comparison of raw blackcurrant mash with 3.58mg anthocyanin/g mash to the left with blackcurrant mash containing 0.42mg anthocyanin/g mash to the right. The white colour at the bottom of the second sample from the left is a magnetic stirrer.

When the anthocyanin content for the first batch was plotted against the colour values some correlations could be seen. For the a -values, regression according to $y=kx^n$ gave the coefficients $k=13.2$ and $n=0.32$ with a R^2 value of 0.88. With the b -values the decrease is started earlier and is more consistent which yields a straight line ($y=kx+m$) with a slope of $k=0.39$ and m -value of 2.69 with a R^2 value of 0.91. In both cases other models were tried as well but these led to the best fit in each of the cases.

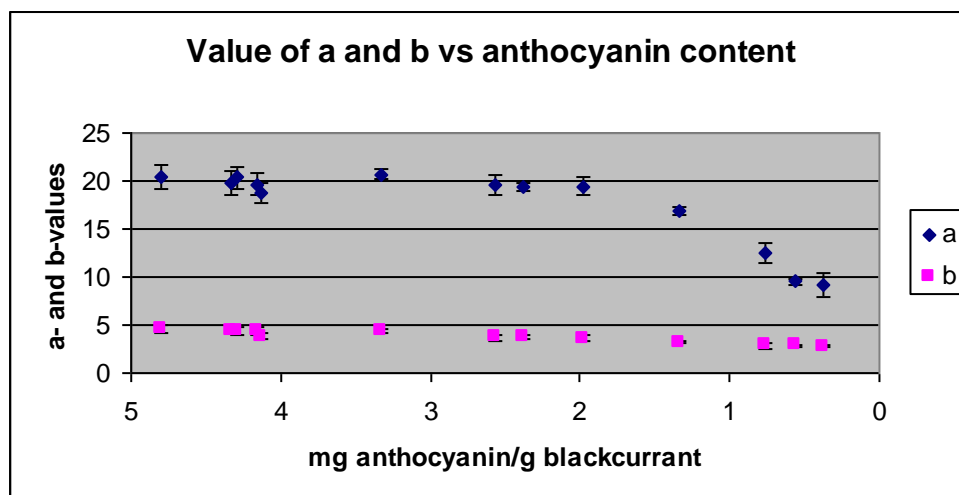


Figure 17 Graph of how the colour values changes with decreasing anthocyanin content in batch 1

Similar measurements were performed on the second batch to investigate if a general model or trend for blackcurrant colour could be obtained. The results looked somewhat similar but the numerical values differed (see fig 17). When using regression on the different values the a -values can be modelled with a similar $y=kx^n$ formula with $k=20.12$ and $n=0.33$ with a R^2 value of 0.94. The b -values does not fit particularly good to any regression model though with the closest being the linear formula $y=1.48x+3.6$ with a R^2 value of 0.76. The next step was to

try to produce a standard curve by choosing treatments that would produce samples with predetermined anthocyanin content and thereby be able to test if the method could be reproduced. These colour values was in the range of the first experiments but failed to give any regression models with a R^2 value of more than 0.81.

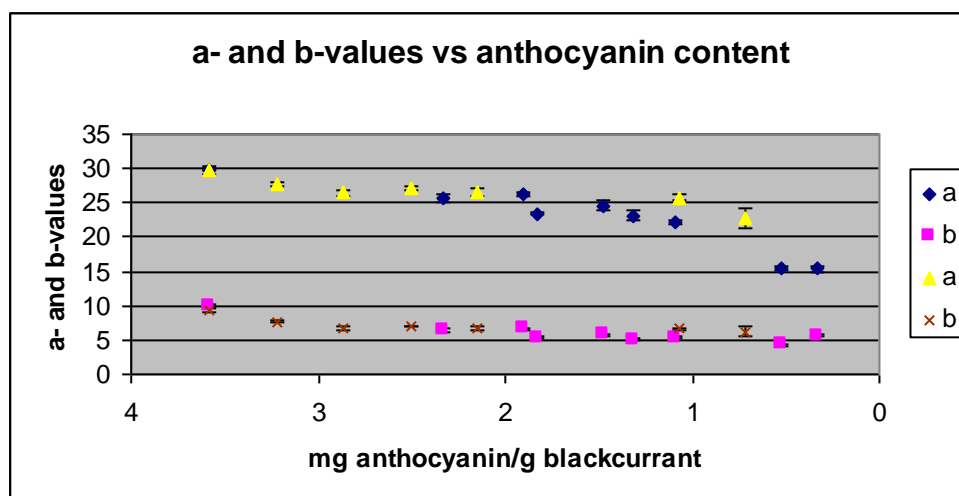


Figure 18 *a* and *b* values from two separate trials on the second batch showing similar behaviour. The blue and purple dots are the first trial while the yellow and brown markings are from the second.

When the same procedure was tried with bilberries it was soon concluded that the method used for blackcurrants was not suitable for the bilberries. The bilberries had too many small dark particles, such as seeds and skin residue, which were big enough to sediment during the measurements but easily passed through the filter used to remove the skin and seeds of blackcurrant. Diluted samples became inhomogeneous, even after 20-fold dilution and it was therefore not possible to obtain reproducible measurements.

The first aim of the colour experiments was to investigate if it was possible at all to measure colour differences due to loss of anthocyanin. The method established managed to detect the colour loss in blackcurrant, which was the main focus of the tests, but not in the bilberries. The second objective was to investigate if there was any correlation between the loss of colour and the loss of anthocyanin. In this case some correlation could be seen as the *a*-value, i.e. redness, decreased somewhat accurate according to the formula $y=kx^n$ where *x* is the anthocyanin content. The yellowness also decreased and got more and more grey over time but no real mathematical correlation could be found that suited both batches.

When the values of *a* and *b* from the 2 different batches were compared it became clear that these values could be used for measuring the total anthocyanin content due to the higher redness and yellowness in batch 2 but with lower anthocyanin content, thus effectively a mathematical correlation impossible. This is illustrated in figure 18 and 19. Another problem, inherited from the first part of the thesis, is that there is no data available on the degradation products of the anthocyanins and hence not their contribution to the colour either.

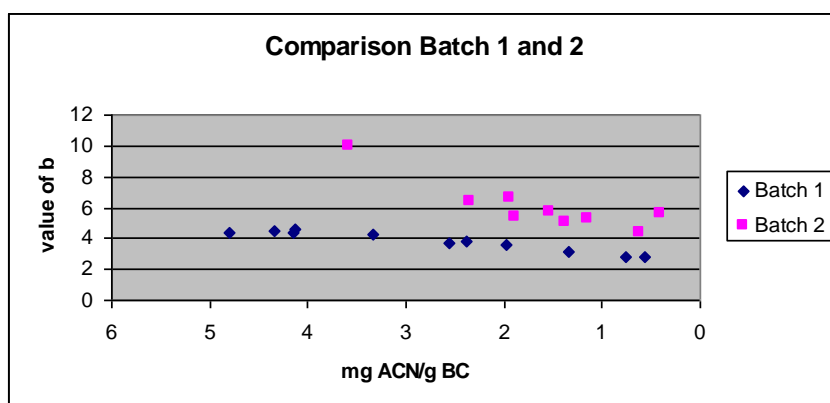


Figure 19 Values of a in the 2 batches of blackcurrants

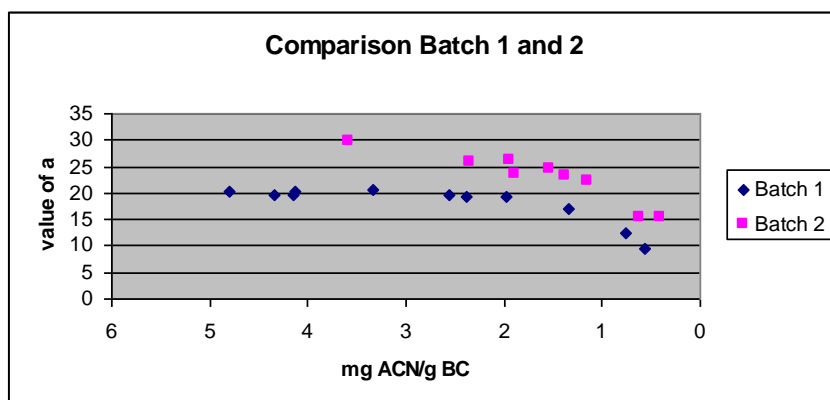


Figure 20 Values of b in the two batches of blackcurrants

A further problem arising from using colourmetry to measure anthocyanin content in a berry is that different anthocyanins give rise to different colours even though present in the same amounts. Cyanidin-3-glucoside is present in most common fruits and berries but it is other anthocyanins normally give the distinguishing colour. Dark purple fruits and berries such as blackcurrants and eggplants normally have high concentrations of delphinidins while bright red fruits and berries such as raspberries and strawberries have a higher proportion of pelargonidin (Wu & Prior 2005). This is illustrated in figure 21 and figure 22 and even if they differ in their total anthocyanin content the difference is even bigger between blackcurrants and bilberries which have similar colours. The uneven distribution of anthocyanins in the fruits, as shown in this thesis for instance, also complicates colour measurements on them. For this reason simple colour measurements, such as the ones tested in this thesis, on complex system like jams and whole berries are only suitable for measuring differences within the same type or batch and not between different species.



Figure 21 Raspberries (*Rubus idaeus*)



Figure 22 Blackcurrants (*Ribes nigrum*)

Rheological measurements

In the rheological tests all samples showed a clear shear thinning behaviour in conformity with most other foodstuffs. The raw blackcurrant samples was both thicker than the bilberry sample as suspected and the first batch was a clearly thicker than the second batch even after the removal of the pomace which might once again indicate a higher concentration of water in the second batch than the first one. The high content of pectin in blackcurrants compared to bilberries might also influence this behaviour even though no sugar was added. The fact that the n value is closer to 1 for the second batch might also indicate this even though they both are far away from being Newtonian fluids like water.

The heating experiments of the first batch shows an increase of the consistency index in all cases. According to Vu *et al.* (2004) heating of fruit and vegetables leads to texture degradation due to pectin degradation. This cannot be seen at all but rather the opposite happens in the first batch. After 10 and 60minutes at 100°C the consistency index has increased from 11.04 to 12.42 and 15.76 respectively while the 90 minutes treatment at 120°C causes a minor increase to 11.38. (Table 1)

The testing on the second batch was supposed to investigate whether the perceived difference during the anthocyanin degradation experiments regarding consistency and the increased amount of juice in the samples with ascorbic acid added to it had any foundation or was just a coincidence. The sample with citric acid could to some extent be used as reference though since it does not affect the anthocyanin content as shown earlier. When comparing these two samples the one with ascorbic acid added is having a lower consistency index and the highest flow behaviour index of any of the samples, 5.66 and 0.412 compared to 7.87 and 0.382. Both of these samples had a lower consistency index than the raw sample but higher than the sample treated at 80°C for 10minutes which has the biggest decrease of any samples, from 8.80 to 4.48. (Table 1) The results from batch 2 are consistent with the proposed theory.

| Sample | K | n | R ² |
|--|-------|-------|----------------|
| Blackcurrants raw Batch 1 | 11.04 | 0.258 | 0.98 |
| Blackcurrants raw Batch 2 | 8.80 | 0.344 | 0.98 |
| Bilberries raw | 6.27 | 0.289 | 0.96 |
| Blackcurrants Batch 2 80°C 10min | 4.48 | 0.382 | 0.97 |
| Blackcurrants Batch 2 80°C 60min 4% CA (w/w) | 7.87 | 0.382 | 0.98 |
| Blackcurrants Batch 2 80°C 60min 4% AA (w/w) | 5.66 | 0.412 | 0.99 |
| Blackcurrants Batch 1 100 °C 10min | 12.42 | 0.316 | 0.97 |
| Blackcurrants Batch 1 100 °C 60min | 15.76 | 0.299 | 0.97 |
| Blackcurrants Batch 1 120 °C 90min | 11.38 | 0.303 | 0.96 |

Table 1

A possible explanation for the increases at higher temperatures is the fact that they yielded more purée and less pomace, i.e. there were less seeds and skin left on the filter from the same amount of berry, possibly due to increased degradation. If more of the pectin is degraded into smaller molecules it might cause the smaller parts to be included in the purée instead of staying in the pomace part and thereby increase the consistency of the purée. This could still

be the case for the longer treatment at 120°C but it might have degraded the cell walls even more and hence a lower consistency index again.

The main problem with these experiments is the fact that it is not rheology of the berries themselves that are being measured but rather the purée that have been extracted. The measuring system used, Bob and Cup, required the removal of seeds and skin from the sample since their inclusion would have made any accurate measurements impossible.

The measuring system using a vane is often used in industry for measurements on fruit and berries but this was not possible during this project. This is because of the large sample volume required by the vane measurement system which was not possible to produce with the experimental equipment used during the project since the equipment was already at maximum capacity when producing samples for the Bob and Cup system. Oscillatory tests could not be used either since it was not possible to keep the purée in place between the cone and plate.

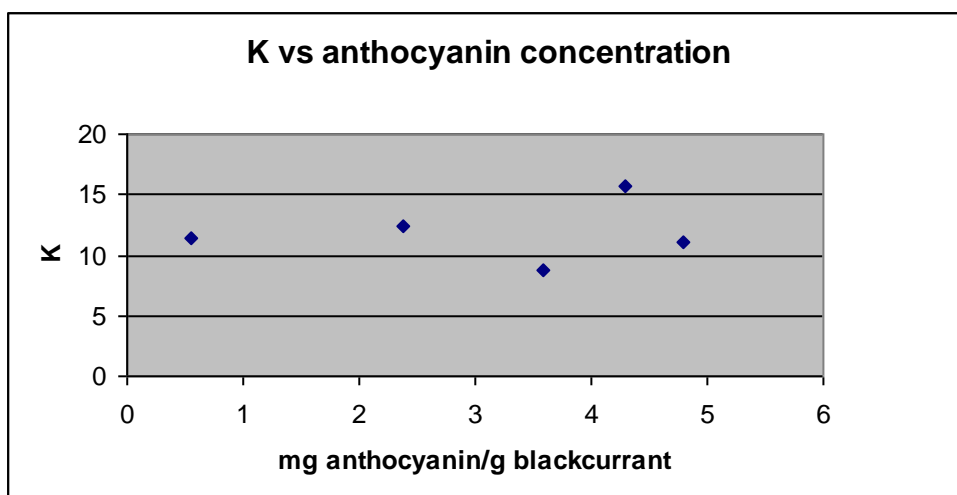


Figure 23 The correlation (or rather lack of) between the consistency and anthocyanin content

It was during the viscosity tests the first batch of berries ran out and it became clear that the berries behaved quite differently from each other. Initial trials with a Bostwick consistometer did not yield any real results since the raw blackcurrant mash at the volumes suitable for treatment simply did not flow after an initial release and 2-5 second movement that is best described as a collapse. Leaving the sample with the hatch opened did not cause any additional movement even if left standing for up to half an hour. A microspatula used for taking samples could also be left standing upright in the beaker with raw blackcurrant mash. This was also true for the second batch of blackcurrants but to a lesser extent in the case of the standing microspatula which could not withstand being tilted slightly as in the first batch. Further experiments with the Bostwick consistometer included using purée and dilution with water but neither was deemed to be useful since the volume of obtained purée was too small for it to flow when the hatch opened and the water dilution required was so big so it had little or no resemblance of the blackcurrant mash of interest.

Earlier studies at SIK had not shown these difficulties in obtaining good purée samples from bilberries for testing in the Stresstech so the behaviour of the bilberry mash in the Bostwick consistometer and ability to keep a microspatula standing was tested as well. The bilberry mash behaved more like a fluid and flowed to the end of the consistometer and could not keep the microspatula upright. The reasons for these differences between the blackcurrants and the

bilberries are not fully known but are believed to be attributed to the higher pectin content and at to some extent the bigger seeds and harder skin of the blackcurrants which was left as pomace after purée extraction. In contrast there was hardly any pomace left from the bilberries after the sieving.

Conclusions and future work

In this master thesis it has been shown that the anthocyanin content in complex food systems, like berries, are degraded exponentially when heated and that the degradation is dependent on the temperature. The degradation was not affected by the initial amount of anthocyanin present in the blackcurrants which indicates that a generalised model for anthocyanin degradation by heating can be used to predict degradation of anthocyanins during processing or storage at elevated temperatures in the future. It was also concluded that the degradation differs between blackcurrants and bilberries but that the results from separate measurements can be used to calculate anthocyanin content in combined products.

Furthermore it was shown that a low pH (below 3) during cooking is important to prevent anthocyanin degradation and that even a small increase in pH to pH 4 before cooking causes irreversible degradation of anthocyanin and a further increase will only exacerbate the process.

Other notable results include the increased degradation of anthocyanin by ascorbic acid in complex systems and protection of anthocyanin by addition of 50% glucose to the mash.

Regarding the colour experiments it has been shown that the colour is affected by the anthocyanin degradation but no clear correlation could be found. More advanced equipment might improve the results but since simplicity and mobility was desired it was not investigated in this project.

The rheological studies showed no correlation between textural properties and anthocyanin content.

For future work it might be interesting to investigate if the antioxidant effect is affected when the anthocyanins are degraded. Other processing methods than heating such as microwave or high pressure treatments could also be investigated for their effects on the anthocyanin content. Furthermore it might be interesting to study individual anthocyanins with HPLC to see if there are any differences in their degradation pattern and stability during processing with different methods.

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Appendix

Appendix 1

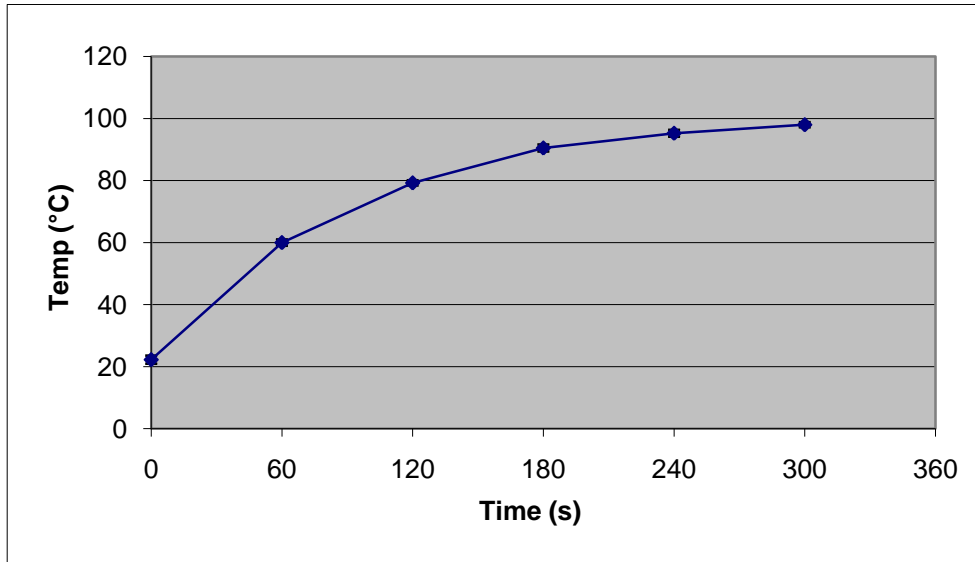


Figure 24

A few experiments were made to determine the time needed to reach the desired temperature in the vials in the glycerol bath. By inserting a thermometer into the vial through the cap the temperature could be measured continuously. For 100 degrees and blackcurrants the profile can be seen in figure 23. The standard deviation during the 4 tests was less than one degree at each 60second interval and can hardly be seen at this scale. In figure 24 the same tests on bilberries is shown and the difference was only 60seconds.

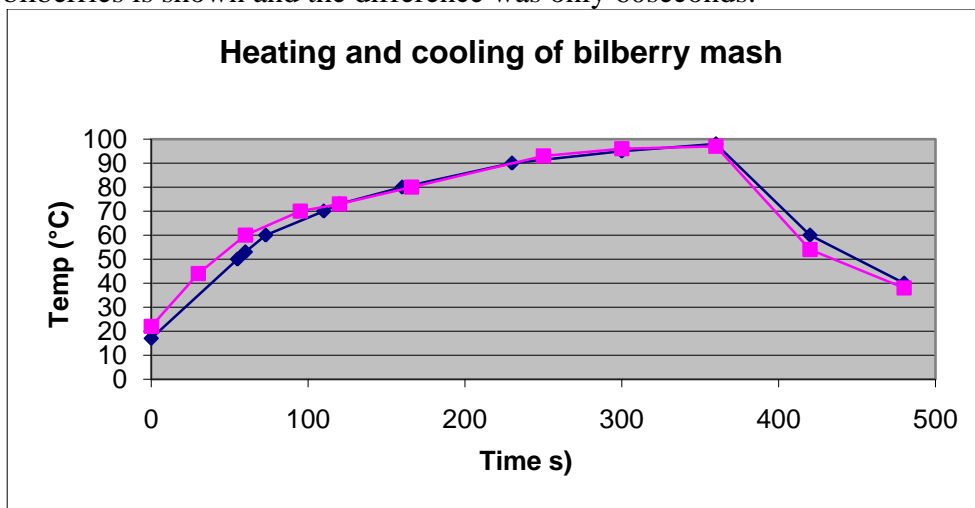


Figure 25 Temperature changes over time in 2 different bilberry mash samples in 100°C glycerol. At t=360s the samples was transferred into ice baths for passive cooling.

Appendix 2

The concentrations and corresponding absorbance values obtained from the standard curve. The R^2 of 0.999 for the entire interval shows that absorbance measurements in the range of 0.4-0.7 can be used to calculate the anthocyanin content in the berries without any problems.

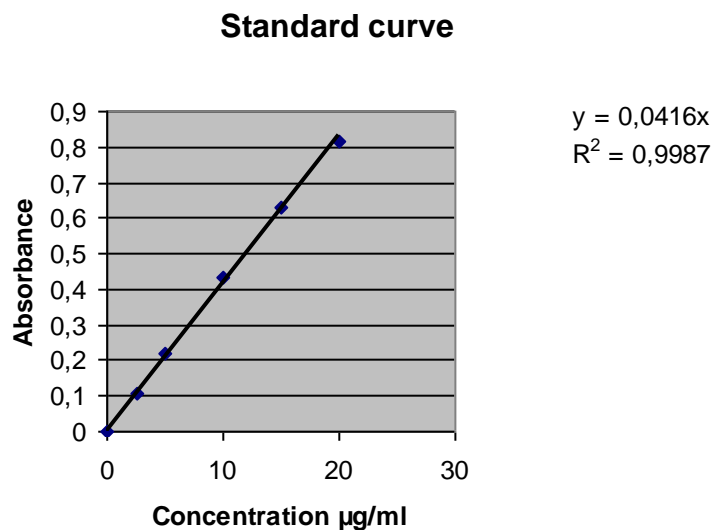


Figure 26 Correlation between the absorbance of a sample and the anthocyanin concentration.