ASTRINGENT FOOD COMPOUNDS AND THEIR INTERACTIONS WITH TASTE PROPERTIES

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ABSTRACT

Astringency is traditionally thought to be induced by plant tannins in foods. Because of this current research concerning the mechanism of astringency is focused on tannin-protein interactions and thus on precipitation, which may be perceived by mechanoreceptors. However, astringency is elicited by a wide range of different phenolic compounds, as well as, some non-phenolic compounds in various foods. Many ellagitannins or smaller compounds that contribute to astringent properties do not interact with salivary proteins and may be directly perceived through some receptors. Generally, the higher degree of polymerization of proanthocyanidins can be associated with more intense astringency. However, the astringent properties of smaller phenolic compounds may not be directly predicted from the structure of a compound, although glycosylation has a significant role. The astringency of organic acids may be directly linked to the perception of sourness, and this increases along with decreasing pH.

Astringency can be divided into different sub-qualities, including even other qualities than traditional mouth-drying, puckering or roughing sensations. Astringency is often accompanied by bitter or sour or both taste properties. The different sub-qualities can be influenced by different astringent compounds. In general, the glycoylation of the phenolic compound results in more velvety and smooth mouthdrying astringency. Flavonol glycosides and other flavonoid compounds and ellagitannins contribute to this velvety mouthdrying astringency. Additionally, they often lack the bitter properties. Proanthocyanidins and phenolic acids elicit more puckering and roughing astringency with some additional bitter properties. Quercetin 3-O-rutinoside, along with other quercetin glycosides, is among the key astringent compounds in black tea and red currants.

In foods, there are always various other additional attributes that are perceived at the same with astringency. Astringent compounds themselves may have other sensory characteristics, such as bitter or sour properties, or they may enhance or suppress other sensory properties. Components contributing to these other properties, such as sugars, may also have similar effects on astringent sensations. Food components eliciting sweetness or fattiness or some polymeric polysaccharides can be used to mask astringent subqualities.

Astringency can generally be referred to as a negative contributor to the liking of various foods. On the other hand, perceptions of astringent properties can vary among individuals. Many genetic factors that influence perceptions of taste properties, such as variations in perceiving a bitter taste or variations in saliva, may also effect the perception of astringency. Individuals who are more sensitive to different sensations may notice the differences between astringent properties more clearly. This may not have effects on the overall perception of astringency. However, in many cases, the liking of astringent foods may need to be learned by repetitive exposure. Astringency is often among the key sensory properties forming the unique overall flavour of certain foods, and therefore it also influences whether or not a food is liked. In many cases, astringency may be an important sub-property
suppressed by other more abundant sensory properties, but it may still have a significant contribution to the overall flavour and thus consumer preferences.

The results of the practical work of this thesis show that the astringent phenolic compounds are mostly located in the skin fractions of black currants, crowberries and bilberries (publications I–III). The skin fractions themselves are rather tasteless. However, the astringent phenolic compounds can be efficiently removed from these skin fractions by consecutive ethanol extractions. Berries contain a wide range of different flavonol glycosides, hydroxycinnamic acid derivatives and anthocyanins and some of them strongly contribute to the different astringent and bitterness properties. Sweetness and sourness are located in the juice fractions along with the majority of sugars and fruit acids. The sweet and sour properties of the juice may be used to mask the astringent and bitterness properties of the extracts. Enzymatic treatments increase the astringent properties and fermented flavour of the black currant juice and decrease sweetness and freshness due to the effects on chemical compositions (IV). Sourness and sweetness are positive contributors to the liking of crowberry and bilberry fractions, whereas bitterness is more negative (V). Some astringent properties in berries are clearly negative factors, whereas some may be more positive. The liking of berries is strongly influenced by various consumer background factors, such as motives and health concerns. The liking of berries and berry fractions may also be affected by genetic factors, such as variations in the gene hTAS2R38, which codes bitter taste receptors (V).
LIST OF ORIGINAL PUBLICATIONS


ABBREVIATIONS

ANOVA  Analysis of variance
AVI     Allele AVI (amino acids alanine, valine, isoleucine) of the gene hTAS2R38
BB      Bilberry
BC      Black currant
CB      Crowberry
DAD     Diode array detector
DOT     Dose over threshold; the ratio of the concentration and taste threshold
ESI-MS  Electron spray ion – mass spectrometer
FCQ     Food choice questionnaire
gLMS    generic labelled magnitude scale
HCA     Hydroxycinnamic acid derivative
HHDP    Hexahydroxydiphenoyl
HPLC    High performance liquid chromatography
hTAS2R38 human gene coding the bitter taste receptor TAS2R38
L-PLS   L-shaped partial least regression
NHTP    Nonahydroxytriphenoyl
PAV     Allele PAV (amino acids proline, alanine, valine) of the gene hTAS2R38
PLS     Partial least regression
PROP    6-n-propylthiouracil
PTC     Phenylthiocarbamide
SFE     Supercritical fluid extraction
TDA     Taste dilution analysis
1 INTRODUCTION

Berries and fruits have been increasingly investigated due to their health related properties. The increased consumption of berries, fruits and vegetables is encouraged in Western diets. Berries and fruits have various beneficial nutritive properties. They are good sources of vitamins, dietary fibre, unsaturated fats and oils and phenolic compounds. Phenolic compounds have various health-promoting properties due to their antioxidant activity. Additionally, phenolic compounds have been reported to possess antimicrobial, anticarcinogenic and antimutagenic properties. However, phenolic compounds have been shown to possess astringent and bitter properties, which may have negative influences on consumption foods rich in phenolic compounds.

Berries often are very sweet and sour with unique aromas. They may have bitter and astringent characteristics, which may contribute to decreased consumer acceptance. They have been used as ingredients in jams, juices and purees in the food industry or used in mixtures with other berries or fruits. In many cases, however, they have been used with added sugars, sweeteners or other food additives to disguise the sour, bitter or astringent off-tastes.

Astringency is a drying, roughing and puckering perception in mouth. Astringency is traditionally induced by various phenolic compounds (e.g. tannins), salts of metallic cations (e.g. aluminium salts, also referred as alums), dehydrating agents (e.g. ethanol) and organic acids (e.g. tartaric and malic acids). The American Society for Testing and Materials (ASTM) defines astringency as “the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins”. Astringency is not confined to certain area in mouth and can be perceived all over the oral epithelia. There are at least three distinct aspects: drying of the mouth, roughing of oral tissues and puckery of drawing sensations felt in the cheeks and muscles of the face. The origin of the term astringency is from a Latin term “ad stringere” meaning “to bind” as astringency is generally believed to be elicited by compounds which bind proteins in saliva and in oral epithelia. Astringents may also be used outside the food context for medicinal purposes due to the shrinking effect on tissues.

The focus of the literature review is on which phenolic compounds and also other non-phenolic compounds contribute to astringency in foods and food materials, how these food components elicit astringent properties, how differently individuals may perceive astringent sensations, how astringent properties interact with taste properties and finally, how astringent properties affect food preferences.

The aim of the thesis work was to study the astringency and other orosensory properties of berries and the key chemical constituents contributing to these properties. The focus was on taste properties such as sweetness, sourness and bitterness, as well as on astringent
mouthfeel and other key characteristics of berries and their fractions. Used materials were fractions of black currants (*Ribes nigrum*), crowberries (*Empetrum nigrum*) and bilberries (*Vaccinium myrtillus*) and black currant juices prepared using different enzymatic and heat treatments. The special aim was to study different astringent sensations (subqualities) contributed by various berry components. Additionally, the effects of enzymes on the sensory profiles and on the sensory-contributing compounds were studied. Finally, the sensory properties and consumer background factors affecting the liking of berries and berry fractions were evaluated using individuals previously genotyped according to variations in the gene *TAS2R38* coding the bitter taste receptor. One special focus was to investigate the roles of sourness, bitterness and two different astringent subqualities in the liking of berry fractions.
2 REVIEW OF THE LITERATURE

2.1 Astringent phenolic compounds in foods

Phenolic compounds can be found in all plant-based foods and food ingredients. Due to the general description of astringency, phenolic compounds in foods may contribute to the astringent properties of the corresponding food materials. These compounds may have other sensory properties, or they may suppress or enhance the properties of other non-phenolic compounds. However, various other non-phenolic compounds in foods may also affect astringency or to the perception of phenolic compounds.

The literature was approached by searching astringency in foods and astringent components, especially phenolic compounds, reported in foods. Furthermore, the reports needed to include sensory evaluations. Thus, only a few food materials are mentioned. The sensory studies described in this chapter have applied sensory evaluations with trained panels using descriptive analyses or taste dilution techniques.

2.1.1 Phenolic compounds found in berries

In this chapter, the phenolic compounds are briefly introduced, concentrating on the compound classes found in berries (Manach et al., 2004; Heinonen et al., 2007). These include various flavonoids, phenolic acids, condensed tannins and hydrolysable tannins. Table 1 summarises flavonol glycosides and hydroxycinnamic acids reported in some Nordic berries. Only a few examples of berries are given in the table. In addition to flavonol glycosides and hydroxycinnamic acids, various gallic and ellagic acid derivatives (hydrolysable tannins) and proanthocyanidins (condensed tannins) have been reported in the berries listed in Table 1. Ellagitannins may occur especially in the genus Rubus, such as in raspberries and cloudberries, and proanthocyanidins in the genera Vaccinium and Ribes.

The structures of phenolic compounds vary from relatively simple to very complex polymers, and these structures may easily be affected by food processing (Cheynier, 2005). The term “polyphenol” is widely used to describe all phenolic compounds. However, the exact definition would require a polymeric structure of phenols (a hydroxyl group attached to an aromatic ring), which is not found in smaller phenolic compounds with only one or two phenyl groups. Similarly, all tannins can be considered as phenolic compounds, but all phenolic compounds are not tannins.
Table 1. Major flavonol glycosides and hydroxycinnamic acid derivatives found in some Nordic berries.

<table>
<thead>
<tr>
<th>Berries</th>
<th>Flavonol glycosides*</th>
<th>Hydroxycinnamic acid (HCA) derivatives*</th>
<th>Literature references</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vaccinium myrtillus</em></td>
<td>bilberry</td>
<td>glucosides, galactosides, arabinosides, xyllosides, rhamnosides, rutinosides and glucuronides of quercetin, myricetin, isorhamnetin and larinctin</td>
<td>Määttä-Riihinen et al., (2004a); Ek et al., (2006); Koponen et al., (2008); Hokkanen et al., (2009); Borges et al., (2010)</td>
</tr>
<tr>
<td><em>Vaccinium vitis-ideae</em></td>
<td>lingonberry</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Vaccinium oxyccocus</em></td>
<td>cranberry</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Vaccinium corymbosum</em></td>
<td>blueberry</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ribes nigrum</em></td>
<td>green currant</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ribes rubrum</em></td>
<td>red currant</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ribes rubrum</em></td>
<td>white currant</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rubus chamaemorus</em></td>
<td>cloudberry</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rubus arcticus</em></td>
<td>arctic bramble</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Hippophaë rhamnoides</em></td>
<td>sea buckthorn</td>
<td>rutinosides, glucosides, sophorosides and rhamnosides of quercetin and isorhamnetin</td>
<td>Rösch et al., (2003); Rösch et al. 2004; Määttä-Riihinen et al., (2004a); Yang et al., (2009)</td>
</tr>
<tr>
<td><em>Empetrum nigrum</em></td>
<td>crowberry</td>
<td>glucosides, galactosides, arabinosides of quercetin and myricetin</td>
<td>Määttä-Riihinen et al., (2004a)</td>
</tr>
<tr>
<td><em>Fragaria x ananassa</em></td>
<td>strawberry</td>
<td>glucuronides of quercetin and kaempferol</td>
<td>Määttä-Riihinen et al., (2004b)</td>
</tr>
</tbody>
</table>

* In addition to the glycosides listed, berries may also have various other glycosides with several sugar moieties (hexoses and/or pentoses) attached to the aglycone. Moreover, flavonol structures may be acylated by hydroxycinnamic acids.
One way to discriminate phenolic compounds is via their biosynthesis (Salminen and Karonen, 2011). Biosynthesis follows either the acetate/malonate or shikimate pathways or a combination of both pathways. Both pathways use products from glycolysis or the oxidative pentose phosphate pathway. The shikimate pathway produces 3-dehydroshikimic acid, which is used in the hydrolysable tannin pathway, and shikimic acid, which is used to form phenylpropanoid precursors of phenolic acids. Importantly, a phenylpropanoid precursor, p-coumaroyl-coenzyme A, is used to make coumaric and caffeic acid derivatives. This precursor is also used, together with malonyl-coenzyme A from the acetate/malonate pathway, to produce flavonoids, condensed tannins and stilbenes, and thereby combining the two pathways. A plant cell does not produce all end products simultaneously; for example, both condensed and hydrolysable tannins. Therefore, they do not accumulate in the same plant tissues. Efficient production of gallic acid from 3-dehydroshikimate acid inhibits the production of shikimate acid and all products using phenylpropanoid precursors.

2.1.1.1 Flavonoids

Flavonoids are the most common group of phenolic compounds in plants (Crozier et al., 2007). All flavonoids contain the same carbon skeleton structure (C6-C3-C6) often forming three ring structures, A, B and C (Figure 1). Due to modifications in the heterocyclic C-ring, flavonoids can be divided into several classes, such as flavonols, flavones, flavan-3-ols, anthocyanins and isoflavones. The common features are a possible double bond between carbons 2 and 3, a possible hydroxyl group on carbon 3 and a possible oxygen on carbon 4 (-OH or =O). Flavonoids usually have sugar or other carbohydrate moieties attached to the aglycone forming flavonoid glycosides. These bonds can be either O-glycosidic or C-glycosidic. Additionally, the carbons in the A- or B-rings can have substituents, such as hydroxyl or methylated hydroxyl groups. Carbons 5 and 7 typically have hydroxyl substituents.
Figure 1. Basic three ring structure (A, B and C in bold) and numbering of the carbons of flavonoid aglycones: a flavonol quercetin (A), a flavone apigenin (B), a flavan-3-ol (+)-gallocatechin (C), an anthocyanidin delphinidin (D) and an isoflavone genistein (E).

Flavonols are the most common group of flavonoids (e.g. quercetin, Figures 1 and 2, and isorhamnetin, Figure 2). They have a double bond between carbons 2 and 3, a carbonyl at carbon 4 and a hydroxyl at carbon 3 in the C-ring. The sugar moiety is commonly attached to the hydroxyl on carbon 3 via an O-glycosidic bond. Flavones are similar to flavonols, but they do not have hydroxyl on carbon 3 (e.g. apigenin, Figure 1). Glycosylation commonly occurs via the hydroxyl on carbon 7. Flavanones lack the hydroxyl on carbon 3 and the double bond at position 2 in the C-ring (e.g. naringenin, Figure 2). The latter feature enables chiral structures for the B-ring.
Flavan-3-ols have the hydroxyl on carbon 3, but they lack the double bond and the oxygen on carbon 4 (e.g. (+)-gallocatechin, Figure 1). They are the monomeric units of proanthocyanidins (Figure 4). They have also chiral structures, but they have two chiral centres, on carbons 2 and 3, enabling four isomeric forms of the basic structure. The more common structures are (+)-catechin and (-)-epicatechin. In the former the B-ring and hydroxyl group are in trans configuration and in the latter, they are in cis configuration. They can also be found as gallates where a gallic acid is esterified to carbon 3. Theaflavins are formed from flavan-3-ols. Anthocyanins have conjugated A- and C-rings and a positive charge in heterocyclic the C-ring; moreover, they lack the oxygen on carbon 4 (e.g. delphinidin, Figure 1, or cyanidin, Figure 2). Isoflavones have the B-ring on carbon 3 of the C-ring instead of carbon 2 (Figure 1).

2.1.1.2 Phenolic acids

Phenolic acids have at least one phenolic group with one carboxyl group. One of the most common is gallic acid (Figure 3) which is rarely found in free form and more commonly as a part of more complex structures, such as in hydrolysable tannins. Gallic acid has a carbon skeleton structure of C6-C1. The other very common group is cinnamic acid derivatives (carbon skeleton structure C6-C3), such as caffeic acid or coumaric acid (Figure 3). Phenolic
acids are often found in esterified forms attached to sugars (usually glucose) or organic acids (e.g. quinic acid in chlorogenic acid; Figure 3). The common modifications are more hydroxylated the aromatic rings and methylation of these hydroxyl groups (e.g. ferulic acid; Figure 3).

Lignans are formed from two C6-C3 structures and have often two rings in their structures. Coumarins are similarly formed from two coumaric acids. Stilbenes, such as resveratrol, have also two aromatic rings in their structures and a C6-C2-C6 carbon skeleton.

![Figure 3. Examples of common phenolic acids: gallic acid (A), p-coumaric acid (B), ferulic acid (C) and chlorogenic acid (3-O-caffeoylquinic acid; D).](image)

2.1.1.3 Condensed and hydrolysable tannins

Tannins are traditionally described as water-soluble phenolic compound polymers with capacity to bind and precipitate proteins and they have molar masses between 500 and 3000 g/mol. Tannins include condensed, hydrolysable and phlorotannins. The latter are rare phenolic compounds found in some brown algae. Proanthocyanidins, often referred to as condensed tannins, are the most common tannins in plants. They have monomeric flavan-3-ols units as common structural units and can be found as oligomers or polymers (Figure 4). The monomers are commonly (+)-catechin or (-)-epicatechin (referred as procyanidins, Figure 4) or (+)-gallocatechin or (-)-epigallocatechin (prodelphinidins, Figure 1). Proanthocyanidin monomers and oligomers are sometimes referred as catechins. Principally, the monomers are linked through the carbons 4 and 8 and more rarely through carbons 4 and 6. In oligomeric and polymeric structures of flavan-3-ols, a third chiral centre, in addition to carbon 2 and 3, is formed at carbon 4.
Figure 4. Examples of proanthocyanidin (condensed tannins) structures: monomeric (+)-catechin (A), (-)-epicatechin (B), epigallocatechin gallate (C), dimeric epicatechin-epicatechin (4–8 bond, known also as procyanidin B2; D) and polymeric proanthocyanidin epicatechin-[epicatechin]_{15} -epicatechin (also with 4–8 bonds; E).
Hydrolysable tannins include gallotannins and ellagitannins. Both are formed through the same route as galloyl groups are attached to central moiety (commonly glucopyranose). When one (monogalloylglucose) to five (pentagalloylglucose, Figure 5) galloyl groups are attached to the central moiety, they are commonly called as simple gallic acid derivatives. If six or more galloyl groups are in the structure, the compounds are called gallotannins and have one or more digalloyl groups. The most common and complex hydrolysable tannins in plants are ellagitannins. They all share a common precursor, pentagalloylglucose, and often contain at least one hexahydroxydiphenoyl (HHDP) group. However, some ellagitannins may be further modified in plant cells or in post-harvest procedures and no longer contain HHDP-groups. Ellagitannins can be divided roughly into six subgroups according to their structures. HHDP esters are the simplest ones (Figure 5). In dehydro-HHDP esters, the HHDP group has been oxidised, and the groups are attached to different hydroxyl groups of the central moiety than in HHDP-esters; therefore, the central moiety is in a different conformation than in HHDP-esters. In modified dehydro-HHPD esters, the HHDP-group is further modified. In C-glycosidic ellagitannins, such as nonahydroxytriphenoyl (NHTP) esters (Figure 6), the central glucose is in an open chain form and often one additional esterified galloyl group is attached to a HHDP-group to form a NHTP-group. Flavonoellagitannins (Figure 6) are hybrids of C-glycosidic ellagitannins and flavonoids (commonly flavan-3-ols). The last subgroup includes various oligomeric ellagitannins, which are commonly oligomers of HHPD esters, but have other subgroups, as well.

Tannic acid, commonly used as a reference compound for astringency in sensory studies, is a mixture of gallotannins with impurities, such as gallic acid and various gallic acid derivatives, although the gallotannins are much rarer in nature than ellagitannins or condensed tannins (Salminen and Karonen, 2011). According to Salminen and Karonen (2011) commercial tannic acids from different suppliers may have mixtures of dozens of

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**Figure 5.** Examples of galloylglucose and ellagitannin structures: pentagalloylglucose (A) and HHDP ester pedunculagin (B).
polyphenols and have significantly different compositions. Even separate tannic acid lots from the same supplier with the same product number may have significantly different compositions.

2.1.2 Astringent compounds in wine

The astringent properties of wines and the corresponding grapes have been and are intensively studied food materials. The astringency of red wine can be divided into a wide range of different sensations with over 30 different descriptors or subqualities (Gawel et al., 2000). Puckering, drying and roughing are the most commonly used descriptors for astringency in the literature, but often the attribute is described only as astringency. Table 2 summarises some of the subqualities used for wines and foods in the literature. Many of those qualities were based on descriptions by Gawel et al. (2000). In a study by Vidal et al. (2003) using various wine-like media made from apple, grape seed and skin tannin fractions, the perceived total astringency did not differ significantly among the evaluated samples, but the various sub-qualities differed. The sensory properties of white and red wines are different with different describing attributes (Oberholster et al., 2009). The addition of phenolic compounds (anthocyanins and tannins) from grape pomace to white wine affects the sensory quality resulting in more typical red wine characteristics, but these are not quite the same as the descriptors for red wine. However, astringency was significantly increased with this addition.

The intensity of astringent perception is dependent on time as the intensity increases after ingestion and persists for a long period of time (Bajec and Pickering, 2008). The development of astringency may vary among different beverages due to different contents of compounds contributing to astringency (Valentova et al., 2002). Astringent properties decrease along with ripening of fruits or berries or with ageing in wine (Bajec and Pickering, 2008). The decrease may be due to an increase in pectins and other polysaccharides, which inhibit the perception of astringent polyphenols (Ozawa et al., 1987). Pectins may interfere with the interactions between polyphenols and salivary and epithelium proteins. A decrease in the concentration of ethanol may result in an increase in wine astringency (Fontoin et al., 2008). Though in general, the alcohol concentrations in wines used the study (from 11% to 15%) are not significantly different.

The volatile compounds in wines can affect the perception of astringency (Saenz-Navajas et al., 2010a). The addition of fruity aromas from white wine to the reconstructed wine model decreases the perceived astringency of this solution. This may have been due to fruity aromas are associated and linked to sweetness, which is inversely related to astringency and bitterness. This effect, however, is not significant with red wine aromas, indicating more significant roles of non-volatiles than volatiles in the perception of astringency.
Table 2. Astringent subqualities and their descriptions.

<table>
<thead>
<tr>
<th>Subquality</th>
<th>Description</th>
<th>Reference</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astringency</td>
<td>No specific subqualities reported.</td>
<td>alum / tannic acid</td>
<td>Peleg et al., (1999); Saenz-Navajas et al., (2010b); Valentova et al., (2002)</td>
</tr>
<tr>
<td>Adhesive</td>
<td>A sensation that mouth surfaces are sticking or adhering to one another (notably between front lips and gums).</td>
<td>-</td>
<td>Vidal et al., (2003); (2004a); (2004b)</td>
</tr>
<tr>
<td>Chalky</td>
<td>Feeling of fine particulate matter that mouth movements can displace.</td>
<td>-</td>
<td>Vidal et al., (2003); (2004a); (2004b); (2004c)</td>
</tr>
<tr>
<td>Dry</td>
<td>Feeling of desiccation or lack of lubrication in the mouth.</td>
<td>-</td>
<td>Vidal et al., (2003); (2004a); (2004b); (2004c)</td>
</tr>
<tr>
<td></td>
<td>Lack of lubrication or moistness resulting in increased friction between oral surfaces.</td>
<td>AIS0₂</td>
<td>Oberholster et al., (2009)</td>
</tr>
<tr>
<td>Mouth-coating</td>
<td>Impression of depth of the coating film that adheres to the mouth surfaces.</td>
<td>various fabrics</td>
<td>Pickering and Robert, (2006)</td>
</tr>
<tr>
<td>Prickling</td>
<td>A tingling feeling sensed by the tongue typically associated with slightly carbonated soft drinks.</td>
<td>-</td>
<td>De Wijk et al., (2003)</td>
</tr>
<tr>
<td>Puckering</td>
<td>A reflexive action of cheek surfaces being brought together and released in an attempt to lubricate mouth surfaces.</td>
<td>-</td>
<td>Vidal et al., (2003); (2004a); (2004b)</td>
</tr>
<tr>
<td></td>
<td>Puckering and roughing astringency.</td>
<td>tannic acid</td>
<td>Scharbert et al., (2004b); Stark et al., (2005); Hufnagel and Hofmann, (2008); Stark et al., 2010</td>
</tr>
<tr>
<td>Roughness</td>
<td>A roughening sensation felt on mouth surfaces when the different surfaces come into contact with each other.</td>
<td>-</td>
<td>Vidal et al., (2004c)</td>
</tr>
<tr>
<td></td>
<td>Roughness sensed on teeth, palate and tongue typically caused by products like walnut, spinach, and wine.</td>
<td>-</td>
<td>De Wijk et al., (2003)</td>
</tr>
<tr>
<td>Smoothness, fine</td>
<td>Fine texture, related to the feel of silk cloth.</td>
<td>various fabrics and materials</td>
<td>Vidal et al., (2003); (2004a); (2004b); Oberholster et al., (2009)</td>
</tr>
<tr>
<td>Smoothness, coarse</td>
<td>Rough texture, related to the feel of coarser grade emery paper.</td>
<td>various fabrics and materials</td>
<td>Vidal et al., (2003); (2004a); (2004b); Oberholster et al., (2009)</td>
</tr>
<tr>
<td>Velvety</td>
<td>Velvety mouth-drying astringency (smooth and mouth-coating).</td>
<td>quercetin-3-O-rutinoside</td>
<td>Scharbert et al., (2004b); Stark et al., (2005); Hufnagel and Hofmann, (2008); Stark et al., 2010</td>
</tr>
</tbody>
</table>
2.1.2.1 Astringent proanthocyanidins in wine

The key astringent compounds in wines have been reported to be various proanthocyanidins (Arnold et al., 1980; Noble, 1994; Gawel, 1998; Bajec and Pickering, 2008) and phenolic acids (Gawel, 1998). As the polymerisation degree of proanthocyanidins increases, the astringency of the compounds simultaneously increases and bitterness decreases (Peleg et al., 1999). Oligomers are more astringent than monomers, but less bitter. Monomer epicatechin has been reported as more astringent than catechin (Noble, 1994; Kallithraka et al. 1997). The astringency induced by catechin and epicatechin is different than alum, grape seed tannins and tannic acids (Kielhorn and Thorngate, 1999). The latter two are, however, mixtures of phenolic compounds, whereas alum and the monomeric flavan-3-ols are pure compounds. Similarly, wines are mixtures of various phenolic compounds and the total contents and the ratios between various sensory-active compounds may vary according to grape species, growing conditions, climate and production methods among other factors.

During the ageing of wine, the interflavanic carbon-carbon bonds of proanthocyanidins spontaneously cleave resulting in a decrease in proanthocyanidin chain length (Vidal et al., 2002). The resulting carbocations in the wine join with other flavan-3-ols via nucleophilic addition. Simultaneously, the carbocations may also proceed to phlobabhenes (Vidal et al., 2002). The decreasing chain lengths may therefore result in the loss of astringency and an increase in bitterness during ageing. The differences in flavan-3-ol monomers in proanthocyanidins affect the astringent subqualities (Vidal et al., 2003). An increasing degree of galloylation correlates with rougher subqualities (coarse and pucker qualities), but coarseness decreases along with increasing hydroxylation of the B-ring of flavan-3-ol monomers (epigallocatechin). Anthocyanin glycosides in red wine do not contribute to the perceived astringency or bitterness (Vidal et al., 2004a).

Grape seed extracts containing various proanthocyanidins and other phenolic compounds are used in red wine production to enhance the structure and ageing potential. Inclusion of the grape seeds among the berry mash in wine-making results in an increase in the content of proanthocyanidins and astringency (Canals et al., 2007). The elimination of seeds results in a significant decrease of both features and bitterness. According to Vidal et al. (2003), grape seeds have more galloylated proanthocyanidins with less hydroxylated B-rings, while grape skins have proanthocyanidin with more hydroxylated B-rings. Astringent reference substances, such as tannic acid and alum, are perceived as more intense in water solutions than in red wine (Valentova et al., 2002).

2.1.2.2 Other astringent phenolic compounds in wines

In comparison to the condensed tannins, flavonol glycosides have been reported to have more significant roles in wine astringency (Hufnagel and Hofmann, 2008a). Flavonol
glycosides, such as 3-O-glucosides and 3-O-galactosides of quercetin, syringetin and isorhamnetin, have been reported to be astringent at very low detection threshold levels. The phenolic acids in wines, especially hydroxycinnamic and benzoic acid derivatives, have been reported to be more puckering astringent, whereas flavonol glycosides were more velvety astringent (Hufnagel and Hofmann, 2008a). However, all these compounds were perceived only as astringent without bitter properties, while phenolic acid esters were perceived as both astringent and bitter. The condensed tannins (proanthocyanidins) and flavan-3-ol monomers in wine were perceived as astringent and bitter, but at significantly higher thresholds. This study was conducted by the fractionation of wine, evaluating the sensory properties of these fractions, studying the key constituents of the fractions and eventually studying the astringent and bitter properties of individual compounds and their detection thresholds. To overcome problems with fatigue and memorising previous intensities, they used the “half-tongue” test, where they applied one sample (with increasing concentrations) on one side of the tongue and water on the other side. Similar studies have been conducted on various other food matrices as discussed later in the chapter 2.1.

In addition to the previously discussed study, where the compounds were first isolated and then evaluated by a sensory panel, a wine model was reconstructed using the chemical constituents previously detected as sensory active (Hufnagel and Hofmann, 2008b). Astringent, bitter, sweet, sour, umami and salty compounds found in wine were used to reconstruct samples. Omissions of individual compounds or compound classes (using nose clips) were conducted to detect the key constituents forming the sensory profile of wine sample. Dose over threshold (DOT) factors indicate the ratio between the concentration of the compound and its detection threshold. The flavonol glycosides were again found to be the key contributors to velvety and mouth-drying astringent sensations. Their omissions significantly decreased astringency. Especially, syringetin-3-O-glucoside had a very high DOT factor (27.0). The omission of hydroxycinnamic acid derivatives had no significant effects on astringency, but the omission of their esters decreased bitterness. Additionally, the omission of a structurally unidentified polymeric polyphenol fraction (size >5 kDa) significantly reduced puckering astringency. In addition to the various astringent compounds, some of the sour, sweet and bitter compounds had significant impacts on the sensory profile.

Saenz-Navajas et al. (2010b) also reported the significance of other phenolic compounds, especially the roles of phenolic acids as contributors to wine astringency rather than oligomeric proanthocyanidins. This study was also conducted by fractionating wine and evaluating fractions. Vanillin and syringic acids (phenolic acids) and cis-aconitic acid (non-phenolic organic acid) were more important contributors than monomeric to tetrameric catechins (both galloylated and non-galloylated). In a study by Preys et al. (2006) the contents of various phenolic acids correlated with astringency, although the content of gallic acid did not correlate, likely due to being the hydrolysis product of galloylated tannins in the
At the same time, the flavonol aglycones quercetin and myricetin correlated with bitterness.

2.1.2.3 Wines matured in oak barrels

Maturing wines in oak wood barrels affects the phenolic content of the wine as hydrolysable tannins (ellagitannins) are dissolved into the wine and thereby affect the sensory quality (Glabasnia and Hofmann, 2006; Stark et al., 2010). The contents of various ellagitannin monomers, such as castalagin, vescalagin (A, Figure 6), grandinin and roburin E in the red wines studied exceeded the detection threshold (DOT factor clearly above a value of 1) and may contribute to some extent to the overall astringency (Glabasnia and Hofmann, 2006). Flavano-ellagitannins, such as acutissimins A and B and epiacutissimins A and B (Figure 6), found in oak wood matured wines, were also detected as astringent at low thresholds (Stark et al., 2010). However, they were found in low amounts, indicating a less significant contribution to red wine astringency. In comparison, the detection thresholds of puckering astringent flavan-3-ols (catechin and epicatechin) were again notably higher with bitter properties. The perceived astringencies of oak wood ellagitannins were smooth and mouth-drying sensations (Stark et al., 2010), as flavano-ellagitannins were perceived as puckering at higher levels.

![Figure 6](image-url)

**Figure 6.** Examples of ellagitannins. C-glycosidic NHTP ester, vescalagin (A) and flavonoellagitannin, epiacutissimin (B). Substituents R1 and R2 in epiacutissimins A and B are OH- and H- or H- and OH-, respectively (Stark et al., 2010).
Similar ellagitannins with low detection thresholds (castalagin, vescalagin, grandinin and roburin E) may also be found in whiskeys which have been matured in oak wood barrels (Glabasnia and Hofmann, 2006). However, their contents are low resulting in low DOT factors and indicating a less significant contribution to astringency. As whiskeys are often matured in toasted oak wood barrels, the toasting procedure may alter the structures of ellagitannins, leading to the formation of degradation products in whiskey (Glabasnia and Hofmann, 2007). The detection thresholds of these products are somewhat higher than the thresholds of castalagin or vescalagin. The toasting of oak wood may therefore have a reducing effect on astringency as ellagitannins are degraded to less astringent products.

2.1.3 Astringent compounds in tea

Similar to wines, the roles of various phenolic compounds, such as proanthocyanidins, theaflavins and flavonol glycosides, in black tea astringency have been studied (Ding et al., 1992; Scharbert et al., 2004a; Scharbert et al. 2004b). Ding et al. (1992) found that the catechin contents correlated with astringency, whereas no similar correlation was detected with theaflavins. Theaflavins (for example A, Figure 7) in black tea were not significant contributors to astringency as they were found in low amounts, below their detection thresholds in three different tea samples (Scharbert et al., 2004a). Scharbert et al. (2004b) reported that the compounds in all three classes were detected as astringent, but flavonol glycosides were the most abundant contributors.

Figure 7. Structure of theaflavin (A) and theogallin (B, Kaneko et al., 2006). Galloyl groups may be attached to chiral hydroxyl groups of theaflavins (Scharbert et al., 2004a).

Quercetin-3-O-rutinoside was one of the key factors in black tea astringency (Scharbert et al. 2004b). Scharbert et al. (2004b) reported that flavonol O-glycosides (rutinosides, glucosides and other glycosides of quercetin, myricetin and kaempferol) were perceived as astringent at very low levels and were significant contributors to the black tea sensory profile. Additionally, a flavone glycoside, apigenin rhamnosylglucoside, was found to be
astringent at very low levels. Scharbert and Hofmann (2005) further showed the significance of these compounds as their DOT factors were significantly high (over a value of 2) in contrast to various other phenolic compounds. The DOT factor of quercetin-3-O-rutinoside was clearly the highest (over 9000), indicating a strong impact on the sensory profile of black tea. Flavonol glycosides were perceived as more velvety and mouth-drying, while proanthocyanidins and theaflavins were more puckering and rough. Similar to red wine, a black tea model was reconstructed (Scharbert and Hofmann, 2005) using the orosensory-active (astringent, bitter, sweet, sour and umami) chemical constituents. The omission of flavonol glycosides significantly affected the perception of velvety and mouth-drying astringency and lowered the perceived bitterness as well. Omissions of caffeine and catechins also lowered bitterness. Although flavonol glycosides were not perceived as bitter when evaluated after isolation, they had roles in black tea bitterness as they enhanced the bitterness of caffeine (Scharbert and Hofmann, 2005).

Kaneko et al. (2006) reported theogallin (a galloyl glycoside; B in Figure 7) to be one of the astringent constituents in Japanese powdered green tea called mat-cha. This compound, along with the astringents theanine and gallic acid, enhanced the perceived umami taste in mat-cha. Various other astringent constituents were not evaluated in the study as it concentrated more on the umami taste.

2.1.4 Astringent compounds in cocoa

Cocoa used in beverages and confectionary products often has naturally bitter and astringent characteristics. Various flavon glycosides in roasted cocoa nibs (Theobroma cacao) contribute to velvety and mouth-drying astringency without bitter properties (Stark et al., 2005). Again, quercetin-3-O-glycosides along with luteolin (O-glycosidic flavone), naringenin (O-glycosidic flavanone) and apigenin (C-glycosidic flavone) glycosides were found to be astringent at very low threshold levels. Additionally, several proanthocyanidins in cocoa contributed to puckering astringency and bitterness. The detection thresholds of these condensed tannins decreased along with the increasing degree of polymerisation (Stark et al., 2005).

Stark and Hofmann (2006) reported that some flavan-3-ols (catechins and epicatechins) were formed non-enzymatically during the alkalisation process of cocoa powder manufacturing and that these had sugar moieties (glucose) connected by C-glycosidic bonds. They had velvety and mouth-drying astringent properties at very low detection thresholds without eliciting any bitter taste. As in comparison, the aglycones catechin and epicatechin were perceived as more puckering astringent with higher detection thresholds and were bitter, as well. The more sugar moieties (glucose units) were connected to the aglycones, the lower detection thresholds for velvety astringency were observed (Stark et al., 2007). Amino acid amines (N-phenylpropenoyl amino acids) also contributed to astringency in
cocoa nibs (Stark and Hofmann, 2005a). The mouthfeel of these compounds have been described as both mouth-drying and puckering. These compounds consisted hydroxycinnamic acid substructures and were synthesised by using caffeic acid as the reagent.

By means of reconstructing a cocoa sample model and omissions of sensory-active compounds, the removal of key astringent compounds in cocoa, especially the velvety astringent ones (flavonol and flavan-3-ol glycosides) and the amino acid amines, had the greatest lowering effect on astringency (Stark et al., 2006). The omission of puckering astringent and bitter compounds (proanthocyanidins) affected bitterness more than astringency. The bitterness in roasted cocoa was mainly due to caffeine, theobromine and some of the 2,5-diketopiperazines (Stark et al., 2006). The latter compounds consist of cyclic structures of two amino acids and also elicit metallic taste properties in cocoa (Stark and Hofmann, 2005b). Only a part of them were perceived as significantly bitter (DOT factors above a value of 1).

2.1.5 Astringent compounds in red currant berries

Only a few studies have been conducted related to astringency-contributing phenolic compounds in berries. Schwarz and Hofmann (2007a) studied the compounds in red currants (Ribes rubrum) contributing to various astringent properties. They found that the key compounds were flavonol glycosides, derivatives hydroxycinnamic acids and various nitrous compounds, such as indoles. The flavonol glycosides contributing to astringency were similar or same as those found in previously mentioned food materials: red wine, black tea infusions and roasted cocoa. The sensations elicited by the studied compounds varied from puckering astringent to mouthdrying astringent, also showing a range of different astringent sensations. Quercetin-3-O-rutinoside was the phenolic compound to elicit astringent properties at the lowest concentration, in addition to various indole compounds. The threshold for perception was at 0.0015 µmol/L for this compound; simultaneously, the thresholds for quercetin-3-O-glucoside and -galactoside were substantially higher (0.7 and 0.5, respectively). The hydroxycinnamic acids in red currants, especially derivatives of coumaric and caffeic acids (O-glycosides), were detected as astringent at similar levels as the flavonol glycosides, also showing their roles in sensory characteristics.

Despite the astringent effects of flavonols and phenolic acids, the indole compounds (for example A and B, Figure 8) and various nitriles (C, Figure 8) have a significant role in the astringent profile of red currants (Schwarz and Hofmann, 2007b). Especially, the indoles associated with sugar moieties have shown significantly lower thresholds for astringent perception. Some of the astringent nitrile compounds also contain a phenyl group (hydroxybenzoic acid) in their structures. Schwarz and Hofmann (2007b) showed the
significant role of glycosylation as the astringency elicited by N- or O-glycosides of indoles had significantly lower detection thresholds than indoles lacking the sugar moiety.

**Figure 8.** Example structures of astringent indoles (A and B) and nitriles (C) in red currants (Schwarz and Hofmann, 2007b).

Similar flavonol glycosides and hydroxycinnamic acids as in red currants or in other aforementioned food materials may be found in other berries, as well (Table 1). In addition to red currants, quercetin-3-O-rutinoside was found in significant amounts in black, green and white currants, lingonberries and sea buckthorns. In most of the flavonol glycosides listed in the table, the glycosidic bond is formed at position 3 of the flavonoid structure, but 7-O-glycosides have also been reported in sea buckthorn (Yang et al., 2009). Although the sensory properties of these compounds are not investigated in the corresponding reports (except for red currants in Schwarz and Hofmann, 2007) the contents of these compounds may have significant roles in berry astringency.
2.1.6 Astringent compounds in spinach

The key astringent compounds in spinach (*Spinacia oleracea*) have been determined as various glycosides and glucuronides of flavonols (Brock and Hofmann, 2008). Especially the glucuronides (for example A and B, Figure 9) and one glycoside of patuletin (C, Figure 9) were found to be astringent at the lowest thresholds. Patuletin compounds were more astringent than spinacetin (D, Figure 9) compounds. The glucuronides were perceived as mouth-drying astringent, whereas flavonol glycosides were more puckering and rough astringent. The aglycones are similar in both compounds, but glucuronic acid moieties are attached to the B-ring via an O-glycosidic bond and sugar moieties are attached to the C-ring in O-glycosides. This difference results in velvety astringency with the former and puckering astringency with the latter. Additionally, a presence of a methylene dioxy bridge in the A-ring of some of the glucuronides lowered the detection threshold in comparison to the glucuronides lacking this bridge.

![Figure 9. Examples of flavonol glucuronides (A and B) and glucosides (C and D) in spinach (Brock and Hofmann, 2008).](image-url)
2.1.7 The role of glycosylation in astringency of phenolic compounds

The glycosylation pattern of flavonol glycosides has a significant role in astringency. Quercetin-3-O-rutinoside had the lowest detection threshold in red currants, whereas for the corresponding glucoside and galactoside it was significantly higher (Schwarz and Hofmann, 2007). In addition to these three glycosides with one or two sugar moieties, the thresholds for quercetin-3-O-dirhamnopyranosylglucoside with three sugar moieties or quercetin-3-O-(dirhamnopyranosylglucoside)-7-O-glucoside with four sugar moieties were even higher. A similar trend was detected for other aglycones in red currants and also in black tea infusions (Scharbert et al., 2004b). Triglycosides with glucose (glucose, rhamnose and glucose) attached to the flavonol aglycone resulted in higher thresholds than the corresponding triglycosides with galactose (galactose, rhamnose and glucose) attached to the aglycone (Scharbert et al., 2004b).

In red currants (Schwarz and Hofmann, 2007), red wine (Hufnagel and Hofmann, 2008a), cocoa (Stark et al., 2005a) and black tea (Scharbert et al., 2004b), quercetin-3-O-glucoside had a slightly higher detection threshold than quercetin-3-O-galactoside. However, the threshold for kaempferol-3-O-galactoside was significantly higher than that of the corresponding glucoside. The aglycone structure also has an impact on detection thresholds. The rutinoside of quercetin had significantly lower thresholds than kaempferol or myricetin (Scharbert et al., 2004b, Schwarz and Hofmann, 2007). The threshold for syringetin-3-O-glucoside was detected as one tenth of the threshold for quercetin-3-O-glucoside (Hufnagel and Hofmann, 2008a).

Further affiriming the importance of glycolysation in polyphenol astringency, ellagitannins with sugar units may be perceived as more intensively astringent (Hofmann et al., 2006). A monomeric ellagitannin, grandinin, has a significantly lower detection threshold than the similar ellagitannin, castalagin, which lacks a pentose moiety, thus indicating the role of C-glycosylation.

Naringin, which is one of the key components influencing the bitterness of grapefruit, is a flavonoid glycoside formed from the aglycone naringenin (flavanone) and a sugar moiety, neohesperidose, linked via an O-glycosidic bond on carbon 7 of the A-ring. In the disaccharide neohesperidose, rhamnose is linked to a glucose through positions 1 and 2, respectively. Narirutin is a very similar flavanone glycoside and has the sugar moiety rutinose, a rhamnose linked to a glucose through positions 1 and 6, respectively. The former is very bitter and the latter is tasteless, indicating again the significant role of glycosylation in sensory perception. Though, no astringent properties have been reported for either of the compounds, they may exhibit some astringent properties at certain concentrations. The astringent properties of the former may also have been masked by bitterness or included in the bitter sensations.
Table 3 summarises some of the phenolic compound groups contributing to astringency and various subqualities in aforementioned food materials (wine, tea, cocoa, spinach and red currants). The sensory profiles, including astringency, were determined by using trained panels. In general, flavonol glycosides (e.g. quercetin compounds), flavan-3-ol glycosides and other phenolic compounds with sugar moieties contribute to more velvety and smooth mouthdrying astringency. Proanthocyanidins and phenolic acids elicit more puckering and roughing astringency. The astringent subqualities of proanthocyanidins may differ along with the degree of polymerisation and with different monomeric units. Some compounds, such as flavonoellagitannins, may have more velvety astringent properties at lower concentrations and puckering astringent properties at higher concentrations.
### Table 3. Summary of phenolic compound contribution to different astringent properties.

<table>
<thead>
<tr>
<th>Phenolic compounds</th>
<th>Astringent subqualities</th>
<th>Literature reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall astringent (no specific subqualities reported)</td>
<td>B</td>
<td>Glabasnia and Hofmann, (2006); Hofmann et al., (2006); Glabasnia and Hofmann, (2007)</td>
</tr>
<tr>
<td>ellagitannins (open chain glucose; their dimers and degradation products)</td>
<td>B</td>
<td>Peleg et al., (1999)</td>
</tr>
<tr>
<td>pentagalloylglucose</td>
<td></td>
<td>Glabasnia and Hofmann, (2006); Kaneko et al., (2006); Saenz-Navajas et al. (2010b)</td>
</tr>
<tr>
<td>phenolic acids</td>
<td></td>
<td>Glabasnia and Hofmann, (2006); Kaneko et al., (2006); Saenz-Navajas et al. (2010b)</td>
</tr>
<tr>
<td>Puckering and roughing astringency</td>
<td>P, R</td>
<td>Vidal et al., 2003</td>
</tr>
<tr>
<td>flavan-3-ols and proanthocyanidins (increasing degree of galloyl groups)</td>
<td>S, P</td>
<td>Stark et al., 2010</td>
</tr>
<tr>
<td>flavonoellagitannins</td>
<td></td>
<td>Brock and Hofmann, (2008)</td>
</tr>
<tr>
<td>phenolic acids</td>
<td></td>
<td>Hufnagel and Hofmann, (2008); Hufnagel and Hofmann, (2008b)</td>
</tr>
<tr>
<td>phenolic acid ethyl esters</td>
<td>P, B</td>
<td>Stark et al., (2004b); Stark et al., (2005); Hufnagel and Hofmann, (2008b)</td>
</tr>
<tr>
<td>flavone glycosides</td>
<td>V, S, MC</td>
<td>Stark et al., (2005)</td>
</tr>
<tr>
<td>flavonoellagitannins</td>
<td>S, P</td>
<td>Stark et al., (2010)</td>
</tr>
<tr>
<td>flavonol glucuronides</td>
<td>M</td>
<td>Brock and Hofmann, (2008)</td>
</tr>
<tr>
<td>flavonone glycosides</td>
<td>V, S, MC</td>
<td>Stark et al., (2005)</td>
</tr>
</tbody>
</table>

Reported subqualities using sensory panels: B = bitterness, M = mouth-drying, MC = mouth-coating, P = puckering, R = roughing, S = silky or smooth, V = velvety. * Astringent at lower and bitter at higher concentrations; ** astringency increases along with degree of polymerisation; *** puckering at higher concentrations.
Table 4. Summary of methods used in some of the sensory studies in the chapter 2.1.

<table>
<thead>
<tr>
<th>Material</th>
<th>Method</th>
<th>Assessors</th>
<th>Literature reference</th>
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<tbody>
<tr>
<td></td>
<td>TDA</td>
<td>12</td>
<td>Glabasnia and Hofmann, (2006)</td>
</tr>
<tr>
<td></td>
<td>TDA</td>
<td>12</td>
<td>Glabasnia and Hofmann, (2007)</td>
</tr>
<tr>
<td></td>
<td>TDA</td>
<td>12</td>
<td>Hofmann et al., (2006)</td>
</tr>
<tr>
<td></td>
<td>Taste profile, TDA</td>
<td>10</td>
<td>Hufnagel and Hofmann, (2008a)</td>
</tr>
<tr>
<td></td>
<td>Taste profile, TDA, reconstruction</td>
<td>10</td>
<td>Hufnagel and Hofmann, (2008b)</td>
</tr>
<tr>
<td></td>
<td>and omission</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Descriptive analysis</td>
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<tr>
<td></td>
<td>TDA</td>
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<td>Stark et al., (2010)</td>
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<td></td>
<td>Time intensity</td>
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<td>TDA</td>
<td>15</td>
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</tr>
<tr>
<td></td>
<td>TDA, omission</td>
<td>15</td>
<td>Scharbert et al., (2004b)</td>
</tr>
<tr>
<td></td>
<td>Taste profile, TDA, reconstruction</td>
<td>15</td>
<td>Scharbert and Hofmann, (2005)</td>
</tr>
<tr>
<td></td>
<td>and omission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocoa</td>
<td>TDA</td>
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<td>Stark and Hofmann, (2005a)</td>
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<td></td>
<td>TDA</td>
<td>12</td>
<td>Stark and Hofmann, (2005b)</td>
</tr>
<tr>
<td></td>
<td>TDA</td>
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<td>Stark et al., (2005)</td>
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<tr>
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<td>Stark and Hofmann, (2006)</td>
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<tr>
<td></td>
<td>Taste profile, TDA, reconstruction</td>
<td>12</td>
<td>Stark et al., (2006)</td>
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<td>and omission</td>
<td></td>
<td>Stark et al., (2007)</td>
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<tr>
<td>Red currant</td>
<td>TDA</td>
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<td>Schwarz and Hofmann, (2007a)</td>
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<tr>
<td></td>
<td>TDA</td>
<td>12</td>
<td>Schwarz and Hofmann, (2007b)</td>
</tr>
</tbody>
</table>

TDA = taste dilution analysis using a “half-tongue” test. In the study of Preys et al. (2006), three different panels were used for different wine samples.

In Table 4 are collected the sensory studies from the Table 3 showing methods and panels they have used. In many cases, the attributes were predetermined to include certain taste and astringent properties. In some cases, nose-clips were used. The taste dilution analyses (TDA) were carried out using a “half-tongue” test, where the diluted sample was applied on the other side of the tongue and water to the other to overcome memory effects of the astringency. Same test was used also for recording dose/response functions, where the individual compounds were compared at their maximum concentrations or they were compared to concentrations of reference compounds.
2.2 Individual differences in astringency perception

2.2.1 Interactions between phenolic compounds and salivary proteins

Due to traditional definitions of the term astringency (to bind) and tannins (polyphenols which precipitate proteins), the mechanism of astringency has been considered to be mediated by binding interactions between tannins and proteins. Then the formed precipitate may be perceived by mechanoreceptors and astringency is then defined as a tactile sensation. However, it has been simultaneously shown that tannins also interact with proteins in the epithelium of the oral cavity and stimulate taste receptors, indicating properties of a taste sensation. Astringency can be elicited by various other non-phenolic compounds, as well. Additionally, many studies have been conducted using tannic acid as the astringent reference, although it is a mixture of various phenolic compounds and not a single compound.

The tactile sensation/taste contradiction has been further discussed in a review article by Bajec and Pickering (2008). As concluded by these authors, astringency may be a result of both taste and tactile sensations. The sense of touch elicited by chemical compounds can be described also as chemesthes. This is generally connected to sensations of hot, cold, pain and irritation. Astringency can also be included among the chemestetic sensations. According to Lawless and Heymann (2010), astringency is a chemically induced tactile sensation. Astringent sensations can be sensed from areas of the mouth, which are lacking in taste receptors.

2.2.1.1 Tannins and salivary proteins

Tannin-protein interactions have been extensively studied as tannins are known to precipitate proteins. The astringent properties of various polyphenols may protect plants from herbivorous animals by making them unpalatable, or the fruit astringency caused by various polyphenols may indicate a less-optimal degree of fruit ripening (Bennick, 2002). Tannins are known to interact with salivary proteins by precipitating them. The interactions between dietary tannins and salivary proteins may be considered as a safety mechanism for not consuming harmful plant secondary metabolites (Shimada, 2006). The precipitating interactions causing the perception of astringency occur through hydrogen bonding or hydrophobic interactions. Salivary and epithelial proteins form complexes with tannins, which can lead to loss of oral lubrication and thereby cause friction on oral epithelia which can be perceived by mechanoreceptors. This leads to a generally accepted conclusion that astringency is a tactile perception rather than a taste property.

Protein-polyphenol interactions have been reviewed many times (Gawel, 1998; Bennick, 2002; Shimada, 2006; Bajec and Pickering, 2008; McRae and Kennedy, 2011) and are therefore only briefly discussed here. The salivary proteins which interact with tannins...
include especially proline-rich proteins (PRP) and histatins (rich in histidine) (Shimada, 2006; Bajec and Pickering, 2008). Protein binding of PRPs has been suggested to occur in three stages (Charlton et al., 2002; Jöbstl et al., 2004). First, tannins bind a randomly coiled protein to form a more compact structure. Then, the tannins on the surface of this complex cross-link with other similar proteins to form dimers, and eventually the dimers aggregate to form larger complexes and precipitate. Formation of a polyphenol-protein complex can be reversible or irreversible (Luck et al., 1994). Salivary proteins show greater affinity to condensed tannins than to hydrolysable tannins (Hofmann et al., 2006). Simultaneously, both condensed and hydrolysable tannins have higher affinities for the PRPs than for bovine serum albumin. The protein affinity and perceived astringency of proanthocyanidins increase with the degree of polymerisation (Arnold et al., 1980; Gawel et al., 1998). This can be observed also with hydrolysable tannins (Luck et al., 1994; Charlton et al. 2002) as the protein precipitation capacity increases with an increasing number of galloyl groups. Polyphenol interactions with various proteins also form hazes, which can be observed by various methods (Horne et al., 2002; Monteleone et al., 2004). The tannic acids, containing mainly gallotannins, also have high protein precipitation capacities. Ellagitannins have a significantly lower precipitation capacity than the galloylglucoses or gallotannins and the capacity is lower for ellagittannins with fewer galloyl groups (Salminen et al., 2011). The protein precipitation capacity increases along with an increasing number of galloyl groups. Simultaneously, as the protein precipitation capacity decreases, the pro-oxidative activity of the hydrolysable tannins increases.

According to Hofmann et al. (2006), only a few studies have been conducted concerning the role of polyphenol structure on protein binding, precipitation or astringency. Most of these studies have concentrated more on the compounds known to precipitate proteins (flavan-3-ols, proanthocyanidins or pentagalloylglucose), and on their interactions with various proteins rather than to study also the astringent properties and mechanisms of other astringent compounds. Proanthocyanidins and pentagalloylglucose were perceived as astringent at slightly higher threshold levels than ellagitannins, castalagin and grandinin (Hofmann et al., 2006). Therefore, the authors suggested that the astringent properties of these ellagitannins are due to binding hydrophobic components of the mouth rather than direct protein binding. The astringent response of tannins may be a combination of protein-binding and hydrophobic interactions with the mouth epithelium. At suprathreshold levels of proanthocyanidins and pentagalloylglucose, the intensity of astringency increased significantly, indicating their astringent properties may be formed after protein binding and complex formation at higher concentrations (Hofmann et al., 2006). Thus for the ellagitannins, lower concentrations are needed to elicit astringency.

Payne et al. (2009) showed that proanthocyanidins bind directly to oral epithelial cells in vitro. The binding is dependent on pH, temperature and concentration. At lower pH (at pH 3.5 rather than 7.0) and at higher temperature (at body temperature rather than room temperature), the binding occurs more intensively. Simultaneously, the detection threshold
for the proanthocyanidins studied decreased along with lowered pH. As some polyphenols bind proteins and form complexes contributing to astringency, their relatively high detection thresholds may be explained by the loss of free compounds in the oral cavity as they have been bound to salivary proteins (Schwarz and Hofmann, 2008). Horne et al. (2002) also showed that an excess level of proteins available in human saliva may protect the oral cavity and lead to a decrease in astringency. Nayak and Carpenter (2008) reported that an increase in salivary proteins inhibited and decreased the perceived astringency. Astringency increased after rinsing the mouth, but decreased after chewing, which induced salivary flow. Rossetti et al. (2009) studied the relationship between the loss of lubrication and perceived astringency using epicatechin and epicatechin gallate; these authors concluded that friction may not be the primary mechanism for the perception of astringency. Both compounds were perceived to be similarly astringent, but had different responses to the loss of salivary lubrication. The latter formed complexes with proteins more efficiently and resulted in the loss of lubrication, but the former did not have this effect.

2.2.1.2 Protein interactions with smaller phenolic compounds

Due to the aforementioned common belief that astringency is primarily caused by interaction of polyphenols and salivary proteins, the mechanism of other smaller astringent compounds has not been well studied. Schwarz and Hofmann (2008) first showed that smaller astringent compounds do not bind with salivary proteins at all. These include, for example, quercetin-3-O-rutinoside (a key astringent compound in black tea infusions and red currants) and carboxymethylindole-1-N-glucoside (a key astringent compound in red currants) which have very low detection thresholds. Astringency perception is not therefore only due to protein binding, complex formation and precipitation. Due to this, Schwarz and Hofmann (2008) suggest that the free compounds (not bound to salivary proteins) are more closely related to astringency. Similarly, Kalithraka et al. (2001) concluded that the free flavan-3-ols in saliva have a more important role in astringency than the precipitated flavan-3-ols. Alternative mechanisms are therefore involved in astringent perceptions, such as receptor activations (Schwarz and Hofmann, 2008). Additionally, these alternatives may be the reason for different astringent sensations, such as velvety astringency caused by flavonol glycosides and puckering astringency caused by proanthocyanidins in red wine (Hufnagel and Hofmann, 2008b). The latter also had some bitter properties, whereas the former was perceived only astringent.

2.2.1.3 Differences in salivary flow

The variation in salivary flow between individuals may influence the perceptions of astringent properties. Individuals with a higher flow rate have been reported to rate
astringency sooner, as less intense and for a shorter period of time than individuals with a lower flow rate using various matrices (Ishikawa and Noble; 1995; Horne et al., 2002; Lesschaeve and Noble, 2005; Bajec and Pickering, 2008). Many studies have failed to find similar results using different matrices, such as organic acid solutions (Bajec and Pickering, 2008). Various foods may have different impacts on the flow rate of saliva as they contain different astringent compounds. Most of the studies concerning flow rate classifications have been carried out using wines, tannic acids or other food matrices rather than individual astringent compounds (Bajec and Pickering, 2008).

2.2.1.4 Variations in polyphenol-protein interactions

Binding of polyphenols with salivary proteins has been used to predict perceived astringency (Llaudy et al., 2004; Monteleone et al., 2004). In a study by Llaudy et al. (2004), polyphenol binding and precipitation with ovalbumin was used to predict the perceived astringency. On the other hand, haze formation in the in vitro-procedure correlated strongly with the perceived astringency and was used to predict the astringency of protein-binding polyphenols, such as tannic acid and grape seed extracts (Monteleone et al., 2004). The predictive model can also be used for more complex polyphenol matrices, such as wines, which contain protein-binding astringent compounds (Condelli et al., 2006). Condelli et al. (2006) further studied the aforementioned correlation to include individually varying factors, such as saliva flow rate, haze formation and protein concentration in their predictions. In their study, they found inverse relations with perceived astringency and saliva flow rate and haze formation. As these factors varied individually, they should be taken into account when choosing assessors to evaluate astringency.

Sensitivity to perceiving astringency may be affected by salivary characteristics varying individually, such salivary flow rate and composition (Dinnella et al., 2009). Subjects with the capability to maintain constant salivary flow were less sensitive to astringency than subjects whose salivary flow was altered by astringent stimuli. Dinnella et al. (2010) further investigated the individual differences in astringency perception by dividing subjects into three groups according to the variations in their salivary protein concentration and haze-forming properties. These factors were measured after masticatory stimulation and then after taste stimulation. The high responding group had the highest amount modifications and significant decrease in their salivary protein concentration, while the low responding group had the lowest amount and no decrease, respectively. Astringency ratings of the high responding group were significantly higher than the moderate and low responding groups. Simultaneously, astringency perception was carried over in the high and moderate groups, where as no variation was detected with the low group subjects.
2.2.2 Sensitivity to PTC and PROP

Sensitivity to perceiving the bitter compounds phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP) vary genetically (Kim et al., 2004). Genetic differences can be located in the gene hTAS2R38 which codes for the corresponding receptor for bitter tasting molecules. This genetic variation, as well as sensitivity to the aforementioned compounds, has been used to divide individuals into three main groups: sensitive bitter tasters, nonsensitive bitter tasters and an intermediate group. In addition to PTC and PROP, three groups have been reported to differ in perceiving various other bitter compounds, bitter vegetables and other taste properties (Sandell and Breslin, 2006; Sandell and Breslin, 2010).

The differences between these groups in perceiving astringency have been studied to some extent. While no studies have been published using groupings based on direct genetic variation, studies using grouping based on the sensitivity to PROP (so-called PROP-status) have showed conflicting results. Noble (1994), Ishikawa and Noble (1995), Smith et al. (1996), Smith and Noble (1998) and Sowalsky and Noble (1998) reported that the PROP-status does not affect perceived astringency. In contrast to these findings, Pickering et al. (2004) reported significant differences between PROP-status groups in perceiving the astringent properties of red wine; the sensitive group perceived the astringency of red wine as more intense than the non-sensitive group. Pickering and Robert (2006) further showed that the former group perceived various sub-qualities of astringency in red wines more intensely, whereas the latter group perceived the total astringency more intensely. They concluded that the sensitive individuals may be better equipped to discriminate and to rate various astringent properties and sub-qualities than non-sensitive individuals. The astringency of alum can also be perceived more intensely by sensitive compared to non-sensitive individuals, especially when using relatively high concentrations (Bajec and Pickering, 2008). In accordance with this, the sensitive individuals rated the intensity of astringency of two different concentrations of alum (a 20-fold difference) as significantly higher than the non-sensitive or intermediate individuals (Bajec and Pickering, 2008b).

Prescott et al. (2004) showed that the sensitivity to PROP is related to sensitivity to irritation in fruit beverages; this may be related to tactile perception of astringency. Astringency was not among the sensory properties investigated in their study, but the irritating sensation was closely related to acids, which are known to elicit astringency in addition to a sour taste. Similarly, in a study by Zhao and Tepper (2007), astringency was not among the properties studied in soft drink models, but they stated that it may be included in the bitter property, which was perceived more intensely by the sensitive group than the non-sensitive group.

2.2.3 Thermal taste and astringency

When a small area on the tongue is heated or cooled some individuals perceive a phantom taste (Cruz and Green, 2000; Bajec and Pickering, 2008b). This occurs most likely on the tip
of the tongue, and approximately 20–50% of the population can be included to this group. In addition to this thermal perception, the sensitive individuals have been reported to rate various taste solutions (sweet, bitter, salty and sour) more intensely than the non-sensitive individuals (Green and George, 2004). Bajec and Pickering (2008b) studied the differences between sensitive and non-sensitive thermal tasters in their perception of astringency. The sensitive thermal tasters rated the various concentrations of alum as more astringent than the non-sensitive thermal tasters. On the other hand, the sensitive individuals did not differ from the non-sensitive subjects in perceiving the irritating properties of capsaicin (Green et al., 2005), which indicates differences between the perceptions of chemesthetic and tactile stimuli.

2.3 Interactions between astringency and taste properties

In contrast to taste properties, the intensity of astringency increases after swallowing, regardless of the concentration of astringent compounds (Ishikawa and Noble, 1995; Bajec and Pickering, 2008). Repeated stimulation with astringent substances may cause buildup of tactile effects as opposed to decrement in taste adaption or desensitisation with chemesthetic substances such as capsaicin (Lawless and Heymann, 2010). In this chapter, taste and other sensations and their relations to astringent characteristics are discussed. The main focus is on the interactions between astringency and taste properties rather than on the interactions between taste properties. As many taste properties can suppress or enhance other properties, they may have effects on the perceived astringency, as well, since astringency has been linked to various other properties.

2.3.1 Bitterness

Bitter taste can be defined as a mechanism for detecting toxins in foods. As briefly mentioned earlier, bitter compounds are detected by various TAS2R receptors which are coded by TAS2R genes (Kim et al., 2003; Kim et al., 2004, Meyerhof et al., 2010). At least 25 different TAS2R receptors are known to detect a huge variety of different bitter toxins and food ingredients. The aforementioned TAS2R38, for example, is associated with detecting compounds containing the thiocyanate (N=C=S) moiety, such as PTC, PROP and glucosinolates (Bufe et al., 2005; Sandell and Breslin, 2006; Sandell and Breslin, 2010). The latter can be found in various vegetables, such as genus Brassica (e.g. broccoli and rutabaga). Although bitter receptors for various isolated compounds have been identified and reported, fewer receptors have been reported for actual bitter components in foods (Hofmann, 2009). Certain bitter phenolic compounds in hops (Humulus lupulus) activate the receptors TAS2R1, -14 and -40 (Intelmann et al., 2009). Tea catechins were recently reported to activate a different bitterness receptor, TAS2R39 (Narukawa et al., 2011).
Various phenolic compounds contributing to astringency also have bitter properties. The monomeric flavan-3-ols, such as catechins and epicatechins, have been reported as more bitter and less astringent, whereas their oligomers and polymers, have been reported as less bitter and more astringent. Bitter and astringent phenolic compounds can be found, for example, in red wine, black tea infusions and cocoa. Most of these compounds can be perceived as astringent at notably lower concentrations and as bitter at higher concentrations. These foods contain various other phenolic compounds, which do not elicit bitter properties at all, and bitter and astringent compounds, which are not referred to as phenolic compounds. The omission of phenolic compound groups from reconstructed food models (Scharbert and Hofmann, 2005; Stark et al., 2006; Hufnagel and Hofmann, 2008b) generally results in the loss of both bitterness and astringency. The non-bitter phenolic compounds perceived as astringent, for example quercetin-3-O-rutinoside, may enhance the intensity of the perceived bitterness of caffeine (an alkaloid) (Scharbert and Hofmann, 2005).

Additionally, various bitter derivatives of phenolic acids can be very strong contributors to the bitterness of roasted coffee. Various caffeic acid, caffeoylquinic acid and feruloylquinic acid derivatives form during thermal treatment and contribute to the bitterness of roasted coffee (Frank et al., 2006; Frank et al., 2007). Some of these phenolic reaction-products elicited some astringent-like sensations, but they were mostly perceived as only bitter. A very high intensity of bitterness may suppress possible and significantly weaker astringent properties. Drewnovski and Gomez-Carneros (2000) reported on the bitterness property of various flavonoids found in a wide range of foods. Although in this review, many of compounds were not referred as astringent (other than flavan-3-ols), they or their glycosides may especially contribute to astringency of foods. Bitterness may be linked to various astringent compounds and foods and, if not separately evaluated, the latter characteristics may be combined with bitter properties.

2.3.2 Sourness
The mechanism for sour taste is not fully understood, as various different mechanisms have been suggested (Kim et al., 2004). For instance, sourness may be perceived through specific ion channels (PKD2L1) located in taste cells (Huang et al., 2006). Sourness can broadly described as a safety mechanism for spoiled (fermented) foods and for optimally ripe foods. However, sourness and acids may be attractive at low concentrations (Kim et al., 2004). Sourness usually decreases during the ripening of fruits and berries along with increasing sweetness. Sour taste has some correlation with pH, specifically with pH of inorganic acids (Kim et al., 2004).
Figure 10. Examples of non-phenolic organic acids in foods: lactic acid (A), citric acid (B), tartaric acid (C), malic acid (D), quinic acid (E) and benzoic acid (F).

Non-phenolic organic (e.g. lactic, citric, tartaric, malic, quinic and acetic acids; Figure 10) and some inorganic acids (e.g. hydrochloric and phosphoric acids) can elicit astringent sensations in addition to a sour taste (Bajec and Pickering, 2008). The astringency caused by acids decreases along with the increasing pH (Lawless et al., 1996). Simultaneously, decreases in the sourness of these compounds also occur, indicating a strong dependence between the properties. Lawless et al. (1996) concluded that the astringency elicited by acids is not due to hydrogen bonding between the acid and oral proteins, but due to the sourness itself. Sowalsky and Noble (1998) reported the astringency of acids being dependent on pH rather than concentration. Lowering pH significantly affected the sourness and astringency of the acids, where as an increased concentration only resulted in an increase in sourness, but not in astringency. Astringency of the acids may, therefore, be due to direct interactions with the epithelial tissues or a pH-dependent denaturation of the lubricating salivary proteins may occur. Lowering pH was also a significant contributor to the astringency of cranberry juice (Peleg and Noble, 1999). Lower pH is the most significant contributor to beer astringency, as well, regardless of the polymerisation degree of the polyphenols (Francois et al., 2006). De Wijk and Prinz (2006) showed that citric and oxalic acids cause aggregations in human saliva and result in an increase in friction.

Berries contain various organic acids, but citric and malic acids can be found in most wild and cultivated berries (Viljakainen et al., 2002). Quinic and benzoic acids are often found, as well, along with various phenolic acids. Sowalsky and Noble (1998) reported that the anion
species is a less relevant contributor to astringency than the pH of the solution. Similarly to astringency, the perceived sourness of various organic acids decreased along with increasing pH (Lugaz et al., 2005). At equivalent pH levels, the perceived sourness of the acids varies, as HCl was less sour than malic, citric, lactic or acetic acids. Acetic acid was perceived as the strongest, but simultaneously it was at a higher concentration at the used pH level than the others. Benzoic acid may elicit pungent and prickling sensory properties in addition to sourness, though its hydroxyl derivatives, such as salicylic and gallic acids, may also elicit astringent sensations (Otero-Losada, 1999).

In sea buckthorn berries, the astringency is partly related to acids and especially to the low pH (Tang et al., 2001; Tiitinen et al., 2005a). Especially, the ratio of sugars and acids in these berries, rather than the total content of acids, is more closely correlated to astringency. Malic acid in sea buckthorn has been reported to especially contribute to the astringency of the berry (Tiitinen et al., 2005a; Tiitinen et al., 2005b). Additionally, the malolactic fermentation process reduced the contents of acids in sea buckthorn berries and thereby increased the pH and at same time decreased the perceived astringency and sourness (Tiitinen et al., 2006). Malolactic fermentation, in which malic acid is converted to lactic acid and carbon dioxide by lactic acid bacteria, is widely used in winemaking to reduce sourness. Regardless of the notable differences in malic acid contents and chemical compositions between different varieties of wine, malolactic fermentation reduces the sourness and astringency properties of most of them (Tiitinen et al., 2007).

Supplementing quinic acid in cranberry juice had no effect on astringency (Peleg and Noble, 1999). Although other organic acids were not investigated in this study, this may indicate that quinic acid does not significantly contribute to the astringency of cranberry. Supplementing citric acid to solutions of phenolic compounds (grape seed tannins, tannic acid, catechin and gallic acid) increased astringency, but the addition of the compounds to an alum solution decreased astringency (Peleg et al., 1998). Various other organic and inorganic acids, which also elicit astringency, caused a similar decreasing effect on the astringency of alum. This indicates different mechanisms of astringency between the alum and phenolic compounds used in that study. Lowering the pH by means of controlling the content of tartaric acid resulted in an increase in the perceived astringency of model wine solutions without affecting the bitterness of the samples (Fontoin et al., 2008). Variations in the content of tartaric acid at the same pH did not affect astringency.

The omission of sour components in red wine (Hufnagel and Hofmann, 2008b) or roasted cocoa (Stark et al., 2006) resulted in significant decreases in perceived sourness. The omission of organic acids from a red wine model solution also resulted in a decrease in puckering astringency, but an increase in velvety astringency (Hufnagel and Hofmann, 2008b). This may indicate a more significant role of organic acids in perception of roughing and puckering astringency rather than in velvety and mouth-drying astringency. Tartaric acid was the most abundant organic acid to elicit a sour taste (with the highest DOT factor) in the
reconstructed red wine model (Hufnagel and Hofmann, 2008b) and, similarly, citric acid was the most abundant in roasted cocoa (Stark et al., 2006).

The sensory attribute of astringency has also been connected with bovine milk. Milk may have a slightly astringent aftertaste (Porubcan and Vickers, 2005). Bayarri et al. (2011) reported the astringency of yoghurts and especially yoghurt-like products to correlate with the bitterness and sourness of the products. Milk and dairy products themselves can be used to mask the astringency and bitterness of polyphenol-rich extracts (Ares et al., 2009). The astringency in milk may be elicited by whey proteins. These proteins elicit astringency in various beverages at relatively low pH (Sano et al., 2005, Beecher et al., 2006). Increasing the whey protein concentration also increased the perceived astringency (Sano et al., 2005). Sano et al. (2005) proposed the mechanism of astringency of whey proteins via interactions with salivary proteins, similarly to polyphenols. Beecher et al. (2006) suggested that astringency is due to interactions between positively charged whey proteins and negatively charged salivary proteins. Vardhanabhuti et al. (2010) reported similar conclusions as protein charges have a significant role in whey protein astringency, especially at low pH. Lee and Vickers (2008), however, proposed that the elicited astringency is due to the acidity of whey proteins and the beverage rather than due to whey proteins themselves. Kelly et al., (2010) concluded that the interactions between whey and salivary proteins are more pH-dependent than interactions between polyphenols and salivary protein and therefore turbidity formation is not equally good feature for measuring whey protein astringency than it is for polyphenol astringency.

2.3.3 Sweetness

The sweet taste receptor is a combination of subunits the TAS1R2 and TAS1R3. Sweetness is closely related to liking from a genetics perspective and is therefore a complex taste quality (Reed and Knäapila, 2010). The purpose of perceiving sweet compounds may be defined as “perceiving energy compounds”, because many naturally sweet compounds can be used for energy.

Berries contain mainly glucose and fructose with notably smaller contents of various other sugars, such as sucrose (Viljakainen et al., 2002). Also, some galactose, xylose and arabinose, as well as sugar alcohols may be found. Sucrose, along with other sugars, may be an efficient masking agent of astringency and bitterness contributed by phenolic compounds (Ares et al., 2009). At relatively high concentrations of an astringent compound (alum or tannic acid), the addition of sucrose had a decreasing effect on astringency, whereas for low concentrations, the same effect was not observed (Brannan et al., 2001a). Additionally, sucrose decreased the bitterness of tannic acid. The addition of sucrose to red wine suppressed the maximum intensity of perceived astringency (Valentova et al., 2002). An
artificial sweetener aspartame did not affect the astringent properties of grape seed tannins (Smith et al., 1996).

Omission of various sweet compounds, such as sugars (aldoses and ketoses) and sugar alcohols (alditols), may have significant effects on several sensory attributes of foods. In a reconstructed model of red wine (Hufnagel and Hofmann, 2008b), the omission of sweet compounds (aldoses, ketoses and alditols) resulted in significant losses of sweetness and mouthfulness and slight increases in both drying and puckering astringent properties, as well as bitterness and sourness. Simultaneously, the omission of key astringent phenolic compounds (non-bitter) resulted in an increase in sweetness. The omission of sweet compounds in black tea (Scharbert and Hofmann, 2005) or in roasted cocoa (Stark et al., 2006) did not significantly affect the sensory properties of these food models. However, sweetness was not among the key sensory characteristics of these foods.

Pectins and various other polysaccharides have decreasing effects on astringency. Polydextrose (Ares et al., 2009) and carboxymethylcellulose (Smith et al., 1996; Troszynska et al., 2010) can be used to reduce the astringent properties of aqueous tannin solutions and polyphenol-rich plant extracts. Arabic, guar and xanthan gums also had a significant capability to reduce astringent properties (Troszynska et al., 2010). According to Smith et al. (1996), the reduction is closely correlated with increased viscosity after the addition of the polysaccharide. The addition did not effect, however, the time to an astringent sensation or on bitterness, although the bitterness was significantly delayed. The study was done using grape seed tannins; Smith and Noble (1998) showed the same reductive effects of a polysaccharide (methyl cellulose) also with the astringents alum and citric acid. Increased viscosity decreased the sourness of citric acid along with astringency.

Rhamnogalacturonan II, an acidic polysaccharide found in red wine and originating from the grape cell walls, has a reductive effect on red wine overall astringency and various sub-qualities, such as chalk, pucker and coarse qualities (Vidal et al., 2004b; Vidal et al., 2004c). Acidic polysaccharides have a more significant effect on decreasing astringency than neutral polysaccharides from the same origin (Vidal et al., 2004b). Similarly, various mannoproteins and arabinogalactoproteins, also originating from the cell walls of grapes, have some reductive effects on wine astringency (Vidal et al., 2004b) and decrease bitterness quite effectively (Vidal et al., 2004c).

The interactions of the polysaccharides with astringent compounds may prevent the latter compounds from binding to the salivary or epithelium proteins or the polysaccharides may as well interact with proteins rather than with astringent compounds (Carvalho et al., 2006; Troszynska et al., 2010). However, the significant differences in chemical structures of different polysaccharides can make the interpretations of the interactions more difficult. The reductive effect may occur due to absorbance of astringent phenolic compounds onto surface of the polysaccharides (Troszynska et al., 2010).
Pectin (Colonna et al., 2004) and carboxymethylcellulose (Brannan et al., 2001b; Colonna et al., 2004) can be used to reduce the carry-over effect of astringency and rinse the oral cavity after the consumption of astringent foods. Crackers (non-salted and rather tasteless) are also efficient palate cleansers to reduce astringent sensations after consumption (Brannan et al., 2001b; Ross et al., 2007). However, Lee and Vickers (2010) have suggested that plain water or nothing is a better cleansing method when evaluating the sensory differences in astringency, regardless of the less efficient cleansing capability of water. Various other cleansers may be good palate cleansers, but they may affect the astringency of the next evaluated sample.

2.3.4 Alums, other salts and metallic sensation

Aluminium sulphates, also referred as alums, elicit strong astringent sensations (Bajec and Pickering, 2008). Partly due to the fact that their contribution to astringency was reported as early as the 19th century, they have been intensively used as reference compounds for astringency in sensory studies. The mechanism for alum astringency has been suggested to differ from the mechanism of tannin astringency (Peleg et al., 1998). De Wijk and Prinz (2005) showed that alum does not decrease the viscosity of saliva and thus does not interact with salivary proteins in the same way as tannic acid. They concluded that the mechanism for alum may be due to flocculation rather than precipitation. Alum may form particles with dead cells or other debris which then cause friction. As a very wide range of different astringent compounds can be found in foods and the overall astringency can be divided to several subqualities, alum astringency may be one among the others and the astringent properties may vary among different alums.

Due to different astringent properties between alum and tannic acid, the former has been suggested not to be used interchangeably in studies related to the astringency of phenolic compounds (Peleg et al., 1998). However, Drobna et al. (2004) suggested, in contrast to the previous study, that alum is a better reference for the astringency of black tea as alum has less interfering bitterness than tannic acid and less interfering sourness than various fruit and berry juices.

The main oral sensory property of various zinc salts is astringency with very few taste properties (Keast, 2003). Copper and zinc sulphates have bitter and astringent characteristics, while ferrous sulphate does not have these properties and is perceived as metallic (Lawless et al., 2004). Ferrous sulphate has a strong metallic taste with some astringent properties, and tastes similar to when an iron nail is placed in the mouth (Lawless et al., 2004). The metallic properties were perceivable even with using nose clips due to the astringent properties of the compound. This was further confirmed by Lim and Lawless (2005a), as the astringent properties were less salient than the astringent properties of copper sulphate. The astringent properties of ferrous sulphate were perceivable at higher
concentrations than copper or zinc sulphates (Lawless et al., 2004). Lawless et al. (2004) concluded that metallic taste may have a similar range of overlapping subqualities as astringency.

In addition to astringent properties, zinc sulphate and chloride also have some savoury (umami) properties similar to monosodium glutamate (MSG) (Yang and Lawless, 2005). Zinc salts also differed from calcium and magnesium salts as the former elicited mainly astringency and the latter two mainly tasted bitter (Lim and Lawless, 2005b). Divalent salts other than zinc have some astringent properties, although when the salts were evaluated using nose clips, the astringent characteristics of ferrous, magnesium and calcium compounds were more distinguishable (Lim and Lawless, 2005b). The astringency elicited by zinc sulphates may be caused by interactions with salivary proteins (Hong et al., 2009).

The addition of zinc to taste solutions decreased the perceived sweetness (glucose) and bitterness (quinine-HCl), but had no effect on sourness (citric acid), saltiness (NaCl) or umami (MSG) (Keast, 2003). Zinc sulphate can inhibit the perceived sweetness of various other sweet compounds as well, such as sucrose, fructose, aspartame, acesulfame-K, sorbitol and saccharin (Keast et al., 2004). The sweetness of sodium cyclamate, however, was not affected by zinc sulphate, indicating a different mechanism of sweetness perception for some compounds. According to Keast et al. (2004), the inhibition was due to zinc ions, rather than sulphate ions, as magnesium sulphate did not have similar effects on sweet compounds.

Calcium chloride is perceived mainly as bitter, but it may have also metallic, astringent and irritating properties (Lawless et al., 2003a). The term irritation has been occasionally substituted for the term astringency when the sensory properties of calcium chloride have been studied (Lawless et al., 2003), which indicates some relationship between the two properties. The sensory properties of calcium are suppressed when the cation is combined with larger organic ions, such as lactate or gluconate. Calcium chloride has a slightly attenuating effect on the astringency of citric acid (Lawless et al., 2003b).

2.3.5 Umami

The umami taste can also be described as a savoury or meaty taste. Similar to sweetness, umami may be considered to be a mechanism of energy detection, as various proteins, amino acids and ribonucleotides elicit an umami taste. Additionally, umami is perceived through a similar heterodimeric receptor composed of TAS1R1 and TAS1R3 (Reed and Knaapila, 2010).

As mentioned earlier, various astringent phenolic compounds in Japanese mat-cha tea enhance the umami taste of the tea infusion (Kaneko et al., 2006). Also mentioned earlier, zinc salts have some umami properties in addition to more intensively perceived astringency.
(Yang and Lawless, 2005). Compounds eliciting an umami taste may be found in various foods and food ingredients, but in many astringent materials, umami is not among the main sensory attributes. In red wine (Hufnagel and Hofmann, 2008b), black tea infusions (Scharbert and Hofmann, 2005) and roasted cocoa (Stark et al., 2006), there are some umami contributing compounds, but these are at very low concentrations and do not significantly contribute to the overall sensory perceptions.

Morel mushrooms (*Morchella spp.*), which are widely used in soups and sauces, have strong umami-tasting properties and some mouth-drying properties (Rotzoll et al., 2005). Although the various fractions made from mushrooms had some astringent mouth-drying properties, the isolated compounds (amino acids, nucleotides and carbohydrates) had more umami, sweet, sour and bitter properties. γ-Aminobutyric acid has been identified as a mouth-drying compound in mushroom fractions with a relatively low detection threshold (0.02 mmol/L). In addition to mouth-drying properties, the compound had some sour and mouth-coating properties, as well. The compound was sourer at low pH and more mouth-drying at higher pH. In this study, the same reference was used for the astringency and mouth-drying properties indicating a close relation between the two, separately used attributes. Chicken broth also induces a very strong umami taste (Dunkel and Hofmann, 2009). Some β-alanyl peptides in the broth elicited a slightly sour taste and somewhat astringent properties when tasted individually. In beef broth, similar β-alanyl peptides along with some amino acid derivatives generated in the Maillard reaction contributed to mouth-drying properties (Sonntag et al., 2010).

2.3.6 Texture and kokumi

As astringency may have various different sub-qualities, some of them may be considered as textural attributes, such as roughness and chalkiness. The tactile perception of astringency may be caused by an increase in physical friction. Foods with fatty or creamy properties may lubricate the oral cavity and thus mask the astringent properties (De Wijk et al., 2003a). The masking of astringency (and bitterness) may be one reason for adding milk to tea or coffee. Similarly, astringent particles may suppress creaminess, fattiness and slipperiness (De Wijk and Prinz, 2006), and sharp particles cause more friction than rounder particles (De Wijk and Prinz, 2005). High friction and thus rough and/or mouth-drying astringent properties are more related to semi-solid foods than solid foods (Prinz et al., 2007). Bite sizes may affect perceived astringency (De Wijk et al., 2003b). With bigger bite sizes of dessert custards, the astringent properties decreased when compared to smaller bites, but the astringency remained along with multiple bites in both cases. Creaminess and temperature attributes had similar impacts, but the fatty afterfeel did not.

Kokumi is a Japanese term used for sensory properties like mouthfulness, richness and complexity without actual taste characteristics (Dunkel et al., 2007). It has also been linked
to a possible calcium tasting receptor (Reed and Knaapila, 2010). Various peptides in edible beans (Dunkel et al., 2007) and in Gouda cheese (Toelstede et al., 2009), as well as oxylipins in avocado (Degenhardt and Hofmann, 2010) contribute to kokumi perception. These studies were conducted by fractionating foods and isolating the key constituents; some of the fractions or compounds had some astringent properties. The glutamyl dipeptides, which contributed to kokumi properties in beans (Phaseolus vulgaris) had some slightly astringent properties at high concentrations. The α- and γ-glutamyl peptides contributing to umami and kokumi in Gouda cheese were slightly astringent at relatively high concentrations (Toelstede et al., 2009). Oleic, capric and caprylic acids (fatty acids) in the same cheese had some rough astringent properties, but at relatively high thresholds and very low DOT factors (Toelstede and Hofmann, 2008). The taste-active compounds in avocado were more bitter and kokumi-enhancing than astringent, but the thermal treatment of the food had a significantly increasing effect on astringency (Degenhardt and Hofmann, 2010).

2.4 Astringency and food preferences

Many consumers are not familiar with the term “astringency”, but they are aware of the mouth-drying, puckering sensations (Childs and Drake, 2010). Astringent properties are often considered as negative contributors to food liking (Lesschaeye and Noble, 2005). Astringency may be accompanied by bitterness, which may similarly be negative factors, at least in foods in which the astringent properties are induced by phenolic compounds. These attributes may be referred to as negative factors in plant-based foods and therefore have a negative influence on consumption, regardless of the possible health benefits (Drewnovski and Gomez-Carneros, 2000). Astringency accompanied by intense sourness may also be a negative factor in liking of berries and affect consumption (Tang et al., 2001). Sweetness is often regarded as a positive driver of liking, opposed to bitterness, sourness and astringency.

Astringency may be a negative or positive driver of liking in various foods. When consumers were divided into clusters according to the liking of various yoghurts, some consumer groups found astringency, bitterness and sourness as negative factors and some found them to be positive factors (Bayarri et al., 2011). Some consumers may also be unaffected by these sensory attributes. Similarly in a study by Geel et al. (2005) investigating the liking of instant coffee drinks, one consumer group preferred astringent, bitter and roasted coffee as another group preferred sweet and less intense coffee. Again, some consumers were not affected by sensory attributes and consumed coffee more out of habit.

The perception of astringency may have an effect on the consumption of phenol-rich foods (Dinnella et al., 2011). Consumers were divided into high, medium and low responding groups according to their salivary characteristics; the high responding group rated the astringency of tannic acid as the most intense. The differences between normal and tannic
acid-spiked fruit and vegetable juices (apple, grape and carrot) were the highest with the high responding group. Simultaneously, the differences between liking of the apple and the spiked apple juices with the latter scoring lower ratings were the highest with this same group. Dinnella et al. (2011) concluded that astringency is a more crucial factor in well-liked foods and has little significance in generally less-liked foods. The high responding group preferred coffee and tea with milk more than the low responding group, which preferred coffee and tea without milk, but similar differences were not observed with various other foods.

The ripening stage affects the astringent properties of various plant-based foods and may influence consumer preferences (Dinnella et al., 2011). Some consumers may like foods of different ripening stages more than others. Similarly, others may prefer various masking agents, such as milk in coffee or tea, while consuming potentially astringent foods (Dinnella et al., 2011). The addition of sucrose to polyphenol-rich beverages may reduce the bitter and astringent properties and positively affect consumer preferences (Jaeger et al., 2009). However, Jaeger et al. (2009) concluded that none of the beverages in the study were still rated as highly accepted by consumers, and the estimates for purchase probability were notably low. They also suggested that adding disclosure information regarding health-related influences may have positive effects on the consumer acceptance. Aroma compounds may have greater influences on consumer acceptance of whey protein beverages than the astringent mouthfeel (Childs and Drake, 2010). Especially, aroma compounds detected retronasally may affect the liking of whey proteins. The consumer preferences of acidic whey protein solutions were higher when using nose-clips than without them, indicating a role of aroma components in disliking whey proteins.

Astringent properties are not positive contributors to quenching thirst, which may be one of the drivers for consuming a beverage (Guinard et al., 1998). Guinard et al. (1998) studied the relationships between thirst-quenching properties and astringency in beer. Astringency correlated negatively with thirst-quenching. They concluded that beers with more malt and therefore more polyphenols may be more astringent and simultaneously be less thirst-quenching. The lack of refreshing effect with astringent drinks depends on the state of oral hydration (Guest et al., 2008). When applying water, astringent or sweet drinks to a dried mouth, there are no differences in sensations, but when applied to a normally hydrated mouth, the astringent drink elicited the least wetting effect.

Astringency in foods may be a negative factor at first, but may be more palatable after several consumptions (Lesschaeve and Noble, 2005). Some astringent foods may be learned to be liked due to the physiological effects. Alcohol in wines or caffeine in coffee and tea may influence further consumption, regardless of the astringent properties. Additionally, these foods may be consumed in social contexts which may encourage further exposure and have positive influence on liking the food or drink. Intrinsic properties of foods, such as sensory characteristics, are not the only factors influencing food preferences. Acceptance of
foods may also be influenced by various extrinsic properties, such as brands, health claims and labels, and consumer background factors, such as traditions, concerns, moods and motives.

2.5 Concluding remarks

Astringency is traditionally thought to be induced by plant tannins in foods. Due to this, the current research concerning the mechanism of astringency has focused on tannin-protein interactions. However, astringency is elicited by a wide range of different phenolic and non-phenolic compounds in various foods. Additionally, to elicit astringent properties, the compound may not need to be defined as “a phenolic compound”. Astringency of condensed tannins may occur due to interactions between salivary and epithelium proteins causing protein precipitation in the oral cavity. These precipitates may be then perceived by mechanoreceptors. Many ellagitannins or smaller compounds contributing to astringent properties do not have these interactions and may be perceived directly through some receptors. Generally, the higher degree of polymerisation of the proanthocyanidins is associated with more intense astringency. However, the astringent properties of smaller phenolic compounds cannot be directly predicted from the structure of the compound, but glycosylation plays a significant role.

Astringency can be divided into different sub-qualities, even beyond the traditional mouth-drying, puckering or roughing sensations. The mouth-drying quality is the most common descriptor (Figure 11). The different subqualities may be influenced by different astringent compounds (summarised in Table 3). Figure 11 summarises the interactions described in this literature review. Astringency may be linked to several different attributes. Astringency is often accompanied by a bitter or sour taste, or both taste properties. Astringent phenolic compounds are often linked to bitterness, although not all of them have bitter properties. Astringency of divalent salts may be accompanied by metallic and bitter sensations. Sugars (sweetness) and fats (fattiness, creaminess) may be used to mask astringency. In foods, there are always several attributes perceived at the same time. Astringent compounds may themselves have other sensory characteristics or they may enhance or suppress other sensory properties. Components contributing to the other properties, such as sugars, may also affect similarly on astringent sensations. Astringency of organic acids may be linked directly to the perception sourness as it increases with decreasing pH.
Figure 11. Summary of the interactions between astringency and other orosensory attributes. Some of the key factors are described next to the arrows and some subqualities are mentioned alongside the corresponding attributes (italics).

Astringency may generally be considered as a negative contributor to liking of various foods, but this may only occur at intense levels of astringency. On the other hand, the perceptions of the astringent properties may vary among individuals. Many genetic factors influencing the perceptions of taste properties, such as variations in perceiving bitter taste or variations in saliva, may also affect perception of astringency. Individuals which are more sensitive to different sensations may more clearly observe the differences between astringent properties, which may not affect the overall perception of astringency. However, in many cases, the liking of astringent foods may need to be learned by repetitive exposure. Astringency can often be among the key sensory properties forming the unique, overall flavor of certain foods and therefore it influences liking as well. In many cases, astringency may be an important sub-property suppressed by other more abundant sensory properties, but it may still have a significant contribution to the overall flavor; its presence may be noticed only when it has been completely removed or masked. All in all, astringency is a very complex mixture of sensations, which needs more research, as well as more precise definitions in the future.
3 AIMS OF THE EXPERIMENTAL STUDY

The overall aim was to study the orosensory characteristics and their interactions in Nordic berries. Additionally, the aim was to identify the effects of fractionation on these characteristics, to identify chemical constituents contributing to them and to study their correlation with liking of berry fractions. The goal was especially to determine the effects and possibilities of fractionation in food applications and to improve the marketability of Nordic berries and their fractions in the food industry.

The first aim was to analyse the non-volatile compounds (sugars, organic fruit acids, flavonoids and phenolic acids) in cultivated black currants (*Ribes nigrum*) and in wild crowberries (*Empetrum nigrum*) and bilberries (*Vaccinium myrtillus*) contributing to taste and flavour, especially to bitterness, sweetness, sourness and various astringent properties. The aim was to determine the locations of these chemical compounds and sensory attributes in berries by fractionating the berries and to study the interactions between the sensory properties (I–III).

The second aim was to study the impacts of enzymatic and heat treatments on the orosensory characteristics of the black currant by focusing on the same sensory properties and non-volatile chemical constituents as in the first aim. The effects were studied by preparing juices using enzymes and heat treatments (IV). Additionally, the aim was to study the effects of storage on the aforementioned characteristics (IV).

The third aim was to study the sensory-chemical factors contributing to liking of berry fractions and to study the individual differences (the *hTAS2R38* genotype grouping and consumer background factors) in berry flavour perception and liking (V).
4 MATERIALS AND METHODS

4.1 Sample preparations

4.1.1 Berry materials

Black currants (BC) were obtained from the southwestern part of Finland grown under controlled and monitored conditions in 2005 (I) and 2007 (IV). Crowberries (CB) were collected from the northern parts of Finland in 2006 (II, V) and bilberries (BB) were collected in different parts of Finland in 2008 (III, V). All berries were stored frozen (-20°C) after picking.

4.1.2 Fractionation of berries

Berries were fractioned before the sensory and chemical analyses in order to determine the key sensory properties of each part of the berry and the essential compounds contributing to them (I–III). A general scheme for the fractionation of berry samples is shown in Figure 12. Fractionations included crushing, juice pressing, ethanol extractions, ethanol removal and supercritical fluid extractions (SFE). Berries were first thawed and crushed and then fractioned by juice pressing followed by consecutive ethanol extractions of the press residue (Residue I). After the extractions and filtration of extracts the ethanol was evaporated and the extracts were dissolved in water (Extracts 1–4). This processing resulted in a number of fractions: juice, press residue, ethanol extracts and a residue after ethanol extractions (Residue II). For black currant and crowberry (I–II), SFE processing of Residue II resulted in another residue (Residue III). Additionally, in the black currant study (I), the first extract was put back to Residue I after the first extraction and ethanol removal. The first extract was first dissolved in water and then added back to the residue. For the bilberry study (III), the combined extract of only two consecutive extractions was dissolved to the juice fraction (juice+extract) according to the ratio of corresponding yields.
Figure 12. Preparation scheme of berry samples (I–III). For each berry material, the scheme was similar with some modifications. Ethanol in the combined extract was evaporated after combining the extracts. For studies III and V the combined extract included only two extractions. Chemical analyses of the extracts were conducted using the subextracts.

For the study combining liking and sensory data (V), the bilberry and crowberry fractions used were juice (J), juice+extract (JE) and the combined extract (E) of two extractions (BBJ and CBJ, BBE and CBE, BBJE and CBJE, respectively).

4.1.3 Enzymatic treatments

The general outline of the sample preparation process in study IV is shown in Figure 13. Berries were first crushed and the enzymes were applied. The doses of enzymes were chosen according to the certain enzymatic activities, although all enzymes had various side activities. The addition of enzymes was followed by incubation, pressing and pasteurisation processes. Three different juices were prepared using different incubation and pasteurisation temperatures (40°C/80°C, 40/90 and 50/90) with only one pectinase enzyme (Biopectinase). Two juices were prepared using Biopectinase and an additional enzyme (Macer, higher glucosidase activity) by using two different dosages at a 100-fold difference (40/80+1 and 40/80+100). At the same time, one juice sample was prepared without
enzymes, but following the heat treatments, one sample was prepared without heat or enzyme treatment resembling the juice fraction in study I. Additionally, the 50/90 juice sample was chosen for the storage trial of six weeks (50/90-0, -3 and -6) along with juice prepared with a mixture of various enzymes (Mix-0, -3 and -6).

Figure 13. Preparation of black currant juices (IV). A total of 12 juice samples were used in the sensory evaluations divided into two trials (Biopectinase + Macer and Storage). Untreated and 50/90 juices were included in both trials.

4.2 Sensory evaluations

All sensory analyses and the hedonic test (I–V) were performed at the sensory laboratory in accordance with the ISO 8589-1988 standard.
4.2.1 Sensory profiles

Sensory profiles of the berry fractions and juices (I–IV) were analysed by generic descriptive analyses with trained panels (Table 5). The assessors were pre-trained to recognise taste samples, to rank the taste solutions and differentiate samples in triangle tests. The general guidelines for the selection, training and monitoring of assessors (ISO 8586–1, 1988) were used. The descriptors were generated following DIS 11035 standards (ISO/DIS 1992) during independent training sessions and the assessors were familiarised to the usage of the attributes (Table 6) and the intensity scale (0 to 10).

For all three berries (I–III) and BC juices (IV), at least sweetness, sourness, bitterness and astringency was used and other attributes varied according to the berry. Samples were evaluated in randomised order during three parallel sessions with the help of anchored reference compounds (Table 6) and by using the Compusense-five data collection software (version 4.6, Compusense, Guelph, ON, Canada).

### Table 5. Samples and the panels in the sensory evaluations.

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample descriptions</th>
<th>Number of samples</th>
<th>Number of attributes</th>
<th>Number of assessors</th>
<th>Women / men</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>BC fractions (juice, residues, extracts)</td>
<td>5+4</td>
<td>8</td>
<td>15</td>
<td>8/7</td>
<td>21–57</td>
</tr>
<tr>
<td>II</td>
<td>CB fractions (juice, residues, extracts)</td>
<td>5+5</td>
<td>8</td>
<td>12</td>
<td>8/4</td>
<td>21–57</td>
</tr>
<tr>
<td>III</td>
<td>BB fractions (juice, residues, extracts)</td>
<td>7</td>
<td>7</td>
<td>15</td>
<td>8/7</td>
<td>21–62</td>
</tr>
<tr>
<td>IV</td>
<td>BC juices (different treatments)</td>
<td>7+7</td>
<td>9</td>
<td>12</td>
<td>6/6</td>
<td>21–62</td>
</tr>
<tr>
<td>V</td>
<td>CB &amp; BB fractions (juices, extracts)</td>
<td>6</td>
<td>4</td>
<td>39</td>
<td>30/9</td>
<td>20–60</td>
</tr>
</tbody>
</table>
Table 6. Sensory attributes and their descriptors with reference samples.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Description</th>
<th>Reference</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total intensity</td>
<td>first impression of flavour</td>
<td>-</td>
<td>I–IV</td>
</tr>
<tr>
<td>Soursness</td>
<td>sour taste</td>
<td>citric acid</td>
<td>I–V</td>
</tr>
<tr>
<td>Sweetness</td>
<td>sweet taste</td>
<td>fructose/ sucrose</td>
<td>I, III–IV / II</td>
</tr>
<tr>
<td>Bitterness</td>
<td>bitter taste</td>
<td>caffeine / caffeine + quinine-HCl / quinine-HCl</td>
<td>I–II / III–IV / V</td>
</tr>
<tr>
<td>Astringency</td>
<td>puckering, drying mouthfeel</td>
<td>NH₄Al(SO₄)₂ / AlSO₄</td>
<td>I / II–IV</td>
</tr>
<tr>
<td>Soft astringency</td>
<td>soft and velvety mouth-drying mouthfeel</td>
<td>NH₄Al(SO₄)₂</td>
<td>V</td>
</tr>
<tr>
<td>Rough astringency</td>
<td>roughing and puckering mouthfeel</td>
<td>AlSO₄</td>
<td>V</td>
</tr>
<tr>
<td>Woody</td>
<td>woody mouthfeel</td>
<td>moistened wooden tongue depressors</td>
<td>II</td>
</tr>
<tr>
<td>Dusty</td>
<td>dusty, drying mouthfeel</td>
<td>a piece of filter paper placed on the tongue</td>
<td>II</td>
</tr>
<tr>
<td>Roundness</td>
<td>compact and multidimensional</td>
<td>-</td>
<td>I, III–IV</td>
</tr>
<tr>
<td>Watery</td>
<td>watery, flat overall expression</td>
<td>-</td>
<td>II</td>
</tr>
<tr>
<td>Frutiness / berriness</td>
<td>berry rich flavour</td>
<td>commercial berry juice</td>
<td>I / III</td>
</tr>
<tr>
<td>Sharpness</td>
<td>sharp, acidic, and tangy mouthfeel</td>
<td>lemon juice + malic acid</td>
<td>I</td>
</tr>
<tr>
<td>Fresh odour</td>
<td>fresh berry odour</td>
<td>crushed berries</td>
<td>IV</td>
</tr>
<tr>
<td>Fermented odour / flavour</td>
<td>fermented stuffy odour / flavour</td>
<td>BC juice stored at RT for weeks after opening (odour only)</td>
<td>IV</td>
</tr>
</tbody>
</table>

4.2.2 The hedonic test, sensory profile and questionnaires

A total of 41 voluntary subjects were recruited for study V. They were prescreened for the hTAS2R38 genotype. Participants from all three groups, PAV/PAV (high sensitive to PTC, n = 12), PAV/AVI (n = 13) and AVI/AVI (insensitive to PTC, n = 14), were chosen to participate in the study. Genotyping of subjects was conducted from the collected buccal cell samples.

All 41 subjects participated in the hedonic test. Samples BBJ, CBJ, BBJE and CBJE were selected. The liking of odour, flavour and appearance was measured using a nine-point balanced hedonic scale. The sample presentation order was randomised among and within assessors. Compusense-five software was used for data collection.
Thirty-nine subjects continued from the hedonic test to the following sensory evaluations (Table 5). The sourness, bitterness and two kinds of mouth-drying, astringent properties of the samples (BBJ, CBJ, BBE, CBE, BBJE and CBJE) were evaluated. The first astringency was soft and velvety and the second was rough and puckering mouthfeel. The intensity of each attribute was scored on a gLMS (general Labelled Magnitude Scale) scale with the help of reference compounds. The scale was from 0 (no sensation) to 94.53 (strongest imaginable sensation of any kind). In addition to the four attributes, the assessors were asked to note other significant attributes, if found, without their intensities. The samples evaluated in triplicate during three sessions. The sample presentation order was randomised among and within assessors. Geometric means with standard errors of the mean (SEM) were used to average all assessors and genotype subgroups.

The sensory panel of 39 assessors continued by filling in three questionnaires: the Food Choice Questionnaire (FCQ; Steptoe et al., 1995), the Concern Scale questionnaire (Kähkönen et al., 1996) and a questionnaire concerning use of berries and berry products. The usage questionnaire included questions about overall use, preferences of berry species and preferences of the forms the berries were consumed in. Subjects were divided into three groups depending on the importance of each food choice motive according to their scale values (subcales of the FCQ and Concern Scale), using the 33rd and 66th percentile points as cut-off points. The importance of each motive for each subject was designated as “low”, “moderate”, or “high”, depending on which third of the panel a subject represented on the scale.

### 4.3 Chemical analyses

Anthocyanins, flavonol glycosides and their aglycones and hydroxycinnamic acid derivatives (HCA) of the berry fractions were analysed by HPLC-DAD and HPLC-ESI-MS (I–IV). For anthocyanin analysis, samples were prepared by extracting the fraction consecutively with acidified methanol and for analysis of other phenolic compounds the extractions were made using ethyl acetate. Additionally, acid hydrolysis was used to verify the aglycone parts of the compounds. The quantitative analyses and preliminary identifications were carried out with reverse phase HPLC-DAD with the help reference compounds and UV-vis spectra. The quantifications were carried out using external standards. The corresponding reference compound was used when commercially available, and for others, the most abundant reference compound in the corresponding phenolic compound class was used. Further identifications were conducted by HPLC-ESI-MS in a mass range of m/z 250–1000 in positive and/or negative mode and with the help of literature references and berry reference materials.

Sugars and organic fruit acids including ascorbic acid were determined as trimethylsilyl derivatives by gas chromatography coupled with a flame ionisation detector (I–IV). Sorbitol and tartaric acid were used as internal standards. Compounds were identified with the help of reference compounds and the literature on these compounds in berries.
Total content of chemical constituents in the residues was calculated mathematically according to four consecutive extractions, forming a descending curve (I–III). The equation of the descending curve ($y = Ae^{kx} + C$) was chosen for simplicity and to adjust $R^2$ values using Origin software (Originlab Corp., Northampton, MA). $A$ (amplitude) and $B$ (decay constant) were constant numbers calculated by the software, and $C$ (offset) was set to zero.

### 4.4 Statistical methods

Differences among sample ratings in sensory attributes (I–V) and in liking (V) were analyzed by analysis of variance (ANOVA) together with suitable post-hoc tests: Tukey’s t-test or Tamhane test test ($p<0.05$). Differences among samples in studies I–IV were analysed by a one-way analysis of variance (ANOVA). In study V, a two-way ANOVA was applied for samples (fixed factor) and genotypes (random factor) in hedonic test results. A three-way mixed ANOVA was applied for samples (fixed factor), sessions and subjects (random factors) in sensory attributes. To test the significance of sample main effect, it was tested against the significant sample×subject interaction. To study the differences between genotype groups in sensory attributes, three-way ANOVA (samples, sessions and subjects nested in genotype groups) was applied. Genotypes were further investigated within samples by applying two-way ANOVA (genotypes and sessions).

To find the relationships between the two data matrices, the partial least squares regression (PLS1 or 2) method was applied to standardised data (I–V). X-variables (predictors) were the chemical compounds and Y-variables (responses) were the sensory properties. Cross-validation was used to estimate the number of principal components for statistically reliable models. PLS regression models were used to predict the liking of the extracts in study V. L-PLS regression (V) was applied to combine chemical-sensory data (X-variables) and subject background data (Z-variables) to explain the liking of samples (Y-variables). For this method, the subject background data were treated as categorical and each of the background variables was converted into a binary (1/0) form. Age, gender and the use data were also categorised into subgroups and into the binary form. In addition to the L-PLS regression, bivariate correlations (Pearson’s correlation) between various groupings (from low to high or from AVI/AVI to PAV/PAV) in the subject background and the liking data were analysed to further verify the result of the regression model. The root mean square error (RMSE) of the cross-validation was used to observe the error in the predictions (representing the error of prediction for any new sample on the same scale).

Statistical analyses were performed using SPSS 14.0 (I–II) and 16.0 (III–V) (SPSS Inc. H, Chicago, IL) and Unscrambler 9.8 (I–IV) and 10.1 (V) (Camo Process AS, Oslo, Norway). Panel performances (I–V) were tested using Quali-Sense (Camo Process AS, Oslo, Norway) based on five different univariate tests investigating sensitivity, reproducibility, panel agreement, cross-over effect and rank correlation.
5 RESULTS

5.1 Sample preparations

The juice pressing procedures were made to separate juice fractions from the skin fractions (Residue I). The main purpose in studies I–III was to collect press residues for ethanol extractions. To do this, all the berry skin fractions were collected and measured gravimetrically and the rest was considered as juice. The juice yields varied significantly among the three berries. The pressing of black currants resulted in very viscous juice with relatively low yields, whereas pressing crowberries and bilberries resulted in significantly higher yields due to lower viscosity (Table 7). At the same time, crowberries and bilberries resulted in lower amounts of press residues than black currants. Treating black currants with enzymes prior to pressing had a significant impact on increasing the yield.

Table 7. Yields of the fractions in three berries (I–III) and enzymatic treatments (IV).

<table>
<thead>
<tr>
<th>Fractions from 1000 g of berries*</th>
<th>BC</th>
<th>CB</th>
<th>BB</th>
<th>BC juices (enzymatic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juice (FW / DW)</td>
<td>740 / 80</td>
<td>850 / 58</td>
<td>850 / 45</td>
<td>800–840 / 180–190</td>
</tr>
<tr>
<td>Residue I (FW / DW)</td>
<td>260 / 90</td>
<td>150 / 77</td>
<td>150 / 55</td>
<td>-</td>
</tr>
<tr>
<td>Extract 1 (DW)</td>
<td>18</td>
<td>13</td>
<td>6.5</td>
<td>-</td>
</tr>
<tr>
<td>Extract 2 (DW)</td>
<td>10</td>
<td>8</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>Extract 3 (DW)</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Extract 4 (DW)</td>
<td>5</td>
<td>2</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>Residue II (DW)</td>
<td>55</td>
<td>45</td>
<td>48</td>
<td>-</td>
</tr>
</tbody>
</table>

* FW = fresh weight (g) / DW = dry weight (g). For enzymatic treatments, only juice yields (FW) were measured.

5.2 Sensory evaluations

5.2.1 Sensory profiles

The sensory profiles consisted of different attributes, but sourness, bitterness and astringency were included in all cases (I–V). Sweetness was included in studies I–IV and in study V, only the significant appearance of sweetness was reported by the panel rather than evaluating the intensity. Studies I, III and IV had the attribute roundness, which was rich, round and multidimensional as opposed to a weak and watery sensation. For study II, the watery attribute was chosen rather than roundness. Two studies contained different types
of tactile attributes: astringency, woodiness and dustiness in study II and mouth-drying and roughing astringencies in study V. Study I had the attribute sharpness, indicating a more irritating sensation distinct from sourness. Some attributes more close to olfactory sensations were also included: fruitiness in I and III, fresh and fermented odours and fermented flavour in IV.

For all three berries (I–III) the juice fraction was the sweetest. For black currant it was significantly the sourest, as well. The press residue fractions were very mild and tasteless as compared to the juice fractions. In CB, Residue I was also perceived as somewhat astringent and bitter, but in BC the sample was sourer and sweeter and in BB the sample was very mild. The extracts, on the other hand, were significantly the most astringent in all cases and for bilberry and crowberry, the extracts were also somewhat bitter. In general, the intensities of the sensory attributes decreased along with the number of extractions. Only the watery attribute in crowberries increased, indicating the loss of other attributes (II). The residues left from the extraction process (Residue II) were significantly milder and more tasteless than the press residue. In the black currant study (I), the effect of ethanol processing on the press residue was studied as the first extract was returned back to the press residue after ethanol removal. The sensory profile of this residue was identical to the press residue. In the crowberry study (II), the sensory profile of the SFE residue was very similar to the ethanol extraction residue; these residues were practically identical. In the bilberry study (III), the first two subextracts were applied to the juice fraction. This supplemented juice sample did not differ in any sensory attribute from the normal juice fraction.

Enzymatic treatments of black currants (IV) resulted in a decrease in freshness and an increase in fermented odour and flavour. There were also significant increases in astringency and sourness and a decrease in sweetness. Storage for zero to six weeks had little impact on the sensory profiles of the juice prepared with enzymes. However, sourness increased in the other sample set (Mix) in the storage trial.

In the univariate three-way mixed ANOVA models, the samples were statistically significant in sourness, bitterness and rough astringency. No significant differences were detected between samples in the attribute soft astringency. An example of the three-way ANOVA model results is shown in Table 8. Significant sample×subject interactions were detected indicating some inconsistency in rating samples within attribute. The interactions were tested against sample main effects (MS (sample)/MS(sample×subject) using their corresponding df) to ensure samples were still significantly different in sourness, bitterness and rough astringency. For example in rough astringency, the ratio of MS (sample) and MS(sample×subject) is 27.07, which is higher than the critical F-value for df(5, 190). In bitterness, sessions were statistically significantly different. The samples in the first session were rated as slightly bitterer than in the latter two sessions.
**Table 8.** Example of univariate three-way mixed ANOVA results in study V.

<table>
<thead>
<tr>
<th>Rough astringency</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>426,742</td>
<td>1</td>
<td>426,742</td>
<td>173,341</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>61,346</td>
<td>24,919</td>
<td>2,462</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample</td>
<td>36,144</td>
<td>5</td>
<td>7,229</td>
<td>24,743</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>7,790</td>
<td>26,664</td>
<td>0,292</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Session</td>
<td>0,997</td>
<td>2</td>
<td>0,498</td>
<td>2,688</td>
<td>0,113</td>
</tr>
<tr>
<td></td>
<td>2,015</td>
<td>10,867</td>
<td>0,185</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subject</td>
<td>80,700</td>
<td>38</td>
<td>2,124</td>
<td>7,430</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>30,512</td>
<td>106,744</td>
<td>0,286</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample × session</td>
<td>1,665</td>
<td>10</td>
<td>0,167</td>
<td>1,178</td>
<td>0,304</td>
</tr>
<tr>
<td></td>
<td>53,709</td>
<td>380</td>
<td>0,141</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample × subject</td>
<td>50,722</td>
<td>190</td>
<td>0,267</td>
<td>1,889</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>53,709</td>
<td>380</td>
<td>0,141</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subject × session</td>
<td>12,177</td>
<td>76</td>
<td>0,160</td>
<td>1,134</td>
<td>0,225</td>
</tr>
<tr>
<td></td>
<td>53,709</td>
<td>380</td>
<td>0,141</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SS = sum of squares; df = degrees of freedom; MS = mean square; F = F-ratio between MS(effect)/MS(error); p = significance.

In general, all juice samples in study V were more sour and less bitter and astringent than the extracts. The bilberry juices were significantly sourer than the crowberry juices. The sourness of bilberry juices was evaluated as “moderate” on average on the gLMS scale and crowberry juices only as “weak”. The crowberry juices were more bitter and astringent. Rough astringency was significantly stronger in the extracts, whereas soft astringency was somewhat stronger in the juices. The individual ratings made by the assessors ranged from “strong” to “barely noticeable”. The differences between genotype groups were statistically significant in all of the four attributes (subjects nested in genotypes); as the AVI/AVI group evaluated the samples as more intense than the PAV/PAV group. The differences were more notable in crowberry samples (differences between genotype groups within sample).
5.2.2 Liking and background data

Of the four juice samples included in the hedonic test (V), two bilberry juices were significantly the most pleasant in odour and flavour. The crowberry juices were the most pleasant in appearance. On a nine-point scale, all samples, except CBJE in liking of flavour, were considered at least as slightly pleasant. In a pair-wise comparison between juices and juice+extracts, the appearance of BBJE was more pleasant than of BBI. The similar comparison between crowberry juices showed a lower liking of flavour in CBJE than in CBJ. No significant differences were detected between subject genotype groups.

The means of all subjects in each subscale were equal or above the average on a seven-point (FCQ) or nine-point scale (Concern Scale). Low, medium and high subgroups of subscales were conducted according to the 33rd and 66th percentiles on the FCQ and Concern Scale. Higher importance of health related issues correlated positively with higher importance of “Mood”, “Natural content”, “Price”, “Weight control” and “Ethical factors”. Higher concern correlated positively with older age (>40) as well as with higher importance of “Weight control” and “Price”. “Sensory appeal” did not correlate with other subscales and was clearly the most important of all the subscales. “Mood” was the only subscale, which correlated with genotype as subjects in the PAV/PAV group valued mood-related issues more than the AVI/AVI group. The liking of odour in the samples BBJ, BBJE and CBJ correlated positively with the subscale “Familiarity”, which itself did not correlate with any other subscale. The subscales “Weight control”, “Sensory”, “Concern” and especially “Health” correlated with the liking of appearance in CBJ and/or CBJE. “Weight control” also correlated with liking of flavour.

Almost 90% of the subjects used berries and/or berry products on a weekly basis and only four of the subjects used berries less frequently. However, only 10 used berries (or berry products) every day. The questions other than those on overall use were concerned with preferences (the top three preferences were chosen) with the possibility of overlap. 28% preferred commercial berries/berry products, 38% homemade and for 34% the origin did not matter. 13% preferred sweetened, 16% unsweetened and for 71% the sweetening did not matter. For only 2 of 39 subjects, the form which they consumed did not matter at all. Bilberries were the most preferred berry species as 69% of the subjects (27/39) preferred bilberries and none preferred crowberries.
5.3 Chemical composition

5.3.1 Phenolic compounds

Due to the lack of reference compounds or literature references, many of the identifications were considered as tentative only. All three berries (BC, CB and BB) had high amounts of anthocyanins and an intense colour. In black currants, there were four major anthocyanins, rutinosides and glucosides of delphinidin and cyanidin found along with traces of other minor anthocyanins. The anthocyanins in bilberries and crowberries consisted mostly of galactosides, glucosides and arabinosides of delphinidin, cyanidin, malvidin, peonidin, and petunidin with the first two sugar moieties and the first three aglycones being the most abundant.

The flavonol glycosides in black currant fractions were rutinosides, glucosides, malonylglucosides and arabinosides of myricetin, quercetin and kaempferol. Bilberries and crowberries consisted mostly of galactosides, glucosides, xylosides arabinosides of myricetin, quercetin, larinictrin, isorhamnetin and syringetin with the first two sugar moieties and the first two aglycones being the most abundant. According to the reference compounds and literature, all flavonoids mentioned above were 3-O-glycosides. HCs in these berries were mostly p-coumaric and caffeic acid derivatives and their sugar conjugates. Some minor compounds (anthocyanins, flavonol glycosides and/or HCs) in all three berries were left unidentified (I–III). Most of the phenolic compounds were concentrated in the press residues of the berries and were extractable with number of consecutive ethanol extractions. In general, the contents were the highest in the first extract when comparing the extracts. However, when dissolved in water, in some cases, the highest contents were in the second extract. The addition of the extracts back into the juice fractions (III, V) increased the contents of these compounds significantly (on average an eight-fold increase; V).

The enzymatic treatments (IV) significantly increased the contents of all phenolic compound classes (anthocyanins, flavonol glycosides and aglycones, HCs). Especially the Macer treatment increased the contents of flavonol aglycones and some HCA degradation products. During storage, anthocyanin contents decreased, but the contents of some flavonol glycosides and their aglycones, as well as the degradation products of HCs increased.

5.3.2 Sugars and organic fruit acids

Glucose and fructose were the two most abundant sugars in all three berries (I–III). A notable content of sucrose was found in black currants (I, IV), as well. Citric, malic and quinic acids were the most abundant organic fruit acids. Citric acid was the most abundant sugar in black currants, quinic acid in crowberries and both of these in bilberries. The
Results

significant majority of these compounds were located in the juice fractions of the berries and the rest, left in the press residue, was extracted mainly in the first ethanol extraction step. When the extracts were applied back into the juice fractions (III, V), the contents of these compounds were the highest in the corresponding samples.

Enzymatic treatments increased the contents of glucose and fructose (IV) in BC juices. In these juices prepared with enzymes or with heat treatments, the contents of sucrose were significantly lower than in the untreated juice. In general, the contents of sugars (other than sucrose) and fruit acids (other than ascorbic acid) were the highest in the juice with highest incubation temperature (sample 90/50 in Figure 13). The storage of BC juices resulted in a decrease in sugars and some fruit acids, especially the Mix juices.

5.4 Combining the data

5.4.1 Compounds contributing to sensory properties

PLS2 regression models were used to combine chemical and sensory data in studies I–IV. Chemical variables were used to explain the variation in sensory data variables. In the BC studies (I, IV) only the first two significant factors of the model were shown, whereas in the CB and BB studies (II–III), the first three factors were shown. Some exclusion of the variables was made to produce more statistically reliable models.

The sugars contributed strongly to the sweetness of the juice fractions (I–III). In black currants, particularly (I, IV), the sugars along with the acids were the most significant contributors to the sensory profile of the juice fraction. Due to the intense sourness along with the majority of the sugars and acids, the juice fraction was excluded from the PLS regression model in study I. In the other two berries (II–III), the acids similarly contributed to sourness, although the juice fractions were not the sourest of the sample fractions. Comparing the three berries, the sugar to acid ratio was the highest in black currant juice which was very sweet and sour and the lowest in crowberry juice which was sweet, but not that sour. Bilberry juice was in between with relatively sweet and somewhat sour characteristics.

Phenolic compounds, especially flavonol glycosides and hydroxycinnamic acid derivatives, correlated strongly with the astringency of the berry fractions. The contents of these compounds contributing to astringency along with perceived astringency declined along with the number of extractions. In the PLS models, some phenolic variables correlated with the astringencies of the extracts; these compounds existed in very small quantities in the corresponding extract samples. The majority of these compounds were flavonol glycosides and their aglycones. However, most of the flavonol glycosides and anthocyanins were found mainly in the press residues (Residue I). The other residues had very few phenolic
compounds left from the extraction processes and they did not contain significant amounts of sensory contributing compounds.

5.4.2 Sensory-chemical factors contributing to liking

Three different PLS1 regression models were created to explain the liking of flavour in berry juice samples (V). The first model was created using sensory data to explain the liking of flavour in berry juice samples. All the models showed significant differences between the bilberry and crowberry samples. Sourness was strongly correlated with flavour liking on the right side of the plot along with the bilberry samples. The model also showed strong correlations between sample CBJ and bitterness and rough astringency. The frequency of sweetness was correlated strongly with CBJ. In general, both astringent subqualities and bitterness were negative factors in the liking of the samples.

For the second model, sensory-chemical variables were used to explain liking of flavour. The contents of total sugars and glucose and the sugar/acid ratio were the highest in crowberry samples and therefore negatively correlated with liking, but were simultaneously distinguished from bitterness, astringency and phenolic compounds. Many phenolic compounds along with soft astringency and sweetness, did not contribute to liking equally strongly. In the third model, chemical variables explained liking with similar trends than in the second model.

The three PLS1 regression models were also made by using only the sensory data for each subject genotype group (V). The most notable difference among the genotype models was for the soft astringency, which was a positive factor for AVI/AVI and a negative for PAV/PAV. Rough astringency was also a more negative driver for PAV/PAV as bitterness was for AVI/AVI.

5.4.3 Predicting the liking of berry extracts

The three models allowed for predicting the liking of flavour for bilberry and crowberry extracts (V). Using only sensory data the predicted values were the lowest for both berry extracts. The predicted values of the first model showed a significant decrease in liking when compared to juice samples. The second model with chemical and sensory data predicted somewhat higher liking ratings. Using only the chemical data (third model) predicted the highest values where a notable increase in liking was predicted. RSME cross-validation values to predict the error in the model with two factors were the lowest for the first model.

Predictions were made for all three genotype groups using the sensory data only. All genotype groups were predicted to rate the flavour liking of berry extracts as more
Results

unpleasant than the juices. However, the predicted ratings were notably lower for the PAV/PAV group than for AVI/AVI group (difference between predicted values were more than errors). This indicates that the PAV/PAV subjects may dislike the extracts more than the AVI/AVI subjects, although both groups dislike the extracts rather than like them.

5.4.4 Consumer background factors contributing to liking

The L-PLS regression plot combined subject background data with the liking of flavour of each subject and the sensory-chemical data of the juice samples. The first nine factors in the model explained 63% of subject flavour liking. The sensory-chemical variables (X) were explained to 100% by the three factors and the background variables were explained to 61% by ten factors. Factor 2 discriminated PAV/AVI from AVI/AVI and Factor 3 discriminated PAV/PAV from AVI/AVI. The division of samples and subjects was more significant between berries (BB or CB) than the juice type (J or JE). On one side of the plot were the subjects who liked the flavour of the bilberry juices. These subjects were less concerned about their health and held health, weight control and ethical issues as less important (low subgroups). On the other hand, on the other side of the plot along with more negative sensory attributes were the more concerned subjects, who considered aforementioned features to be of greater importance.
6 DISCUSSION

6.1 Fractionating berries

The significant trends in studies I–III are shown in Figure 14. The berries were fractioned first by crushing and then by pressing. The resulting juices contained the most sugars and non-phenolic fruit acids along with sweetness and some sourness (Figure 14).

![Diagram showing the process of fractionating berries]

**Figure 14.** Summary of significant trends in studies I–III.

The juice of BC was very sour compared to the juices of CB or BB. The contents of sugars and acids were the highest in BC juice, but the sugar to acid ratio was the highest in CB, which was the least sour of the three juices. Additionally, the BC juice had the attribute of sharpness, which correlated with sourness. This attribute may be an astringent subquality related to acids as astringency is often linked to low pH. Although the pH values of the juice fractions were not analysed in the three studies, the pH of BC juice is known to be relatively low (Zheng et al., 2009).

An aqueous ethanol solution (90%) was chosen for the extraction process. However, acidified ethanol or methanol are known to be more effective solvents for extracting
phenolic compounds from berry press residues (Cacace and Mazza, 2003; Lapornik et al., 2005; Pinelo et al., 2005). In studies I–V, one goal was to keep the processes safe and applicable to food processing. Also, the fractionation process was planned to be relatively simple without many additional phases or ingredients. The dilution of ethanol improves the solubility of phenolic compounds (Cacace and Mazza, 2003; Lapornik et al., 2005). The 90% dilution was to ensure sufficient yield and proper evaporation of the solvent after extraction. All of the ethanol was removed from the extracts before the sensory evaluation and tasting of the fractions.

The juice fractions had low contents of phenolic compounds as compared to the press residues, which had relatively mild orosensory characteristics (Figure 14). The quantitative analyses of compounds in the samples were made for all samples, but juices and extracts were also used to calculate the contents in the residues. The calculated contents were significantly higher in many cases than the measured values. The solvents used in the methods were not able to extract all of the compounds, especially from the residues. The phenolic compounds analysed in these studies were very soluble in ethanol and especially in ethanol-water solutions. The contents of these compounds, as well their astringency, decreased along with the number of extractions. In the CB study two additional tactile attributes were evaluated in the extracts; dusty and woody attributes correlated positively with astringency. These were different astringent mouth-drying properties than the sensation elicited by the astringency reference compound, aluminium sulphate. In the fifth study, astringency was divided into soft, velvety and rough, puckering astringencies. The former was more intense in the juices as was the latter in the extracts. To our knowledge, the orosensory characteristics of other fractions of BC, CB or BB than juices have not been previously published.

The analysed phenolic compounds were anthocyanins, flavonol glycosides and their aglycones and hydroxycinnamic acid derivatives. Some of the phenolic compounds (tentatively identified as flavonol glycosides), which correlated with astringency and/or bitterness where not fully identified in this study. They, as well as, the flavonoid aglycones may exist naturally in the berry fractions or they may result from the extraction process. Flavonol glycosides have previously been linked to more velvety and mouth-drying astringency (Scharbert et al., 2004b; Scharbert and Hofmann, 2005; Stark et al., 2005; Hufnagel and Hofmann, 2008a; Hufnagel and Hofmann, 2008b). Hydroxycinnamic acid derivatives, on the other hand, have been linked to puckering astringency and their ethyl esters are also bitter (Hufnagel and Hofmann, 2008; Hufnagel and Hofmann, 2008b). Additionally, many other components, which were not analysed in the berry fractions in these studies, may contribute to different astringent subqualities. Importantly, various tannins were not further analysed, regardless of their appearance in the HPLC chromatograms. Proanthocyanidins may have contributed to puckering and overall astringency of the berries. They may have a role in the bitterness of the extracts as well. Also, the indoles and nitriles, which were reported to contribute to astringency in red currants (Schwarz and Hofmann, 2007a; Schwarz and Hofmann, 2007b), may exist in black currants and other berries. In spite of astringent and other taste-active
components, the extracts of BC, CB and BB also contained volatile aroma components which were not analysed in these studies.

In Figure 15, the juices and the first extracts from the three berries are combined into the same PLS regression plot. The main difference in the first factor is the difference between the juices and the extracts. The second factor shows the differences between berries in those fractions. It is important note, however, that the fractions were sensory evaluated and chemically analysed separately and this may influence the discrimination of variables in the plot. BC juice was clearly the most intense of the all fractions, as well as the sourest. CB juice lacked sourness, but was somewhat sweet. BB and CB extracts were more astringent than BC extract. BB extract was also bitterer than the other two extracts. However, in the CB study, the astringent tactile sensation was divided into different attributes. Due to the different phenolic profiles of the berries (especially between BC and the other berries), the phenolic compounds are shown only as combined groups in Figure 15. All berries contained quercetin glycosides, which have been reported as astringent (Scharbert et al., 2004b; Stark et al., 2005; Schwarz and Hofmann, 2007a; Hufnagel and Hofmann, 2008a). All berries also contained myricetin glycosides. Syringetin and isorhamnetin glucosides, found in CB and BB samples, have also been reported as astringent in red wine (Hufnagel and Hofmann, 2008a), and kaempferol glucosides, found in BC samples, have been reported as astringent in black tea and red currants (Scharbert et al., 2004b; Stark et al., 2005; Schwarz and Hofmann, 2007a). HCA s and flavonol aglycones may contribute to bitterness along with astringency.

![Figure 15. PLS2 regression plot combining the juices and first extracts from studies I–III. Chemical variables (sugars, fruit acids and phenolic compound groups) were used to explain variance in the sensory data (total intensity of flavour, sweetness, sourness, bitterness and astringency). Only variables which were found in all samples were included. Samples are in bigger font, sensory attributes in italics and chemical variables in normal font.](image-url)
The orosensory properties, especially astringent properties, of anthocyanins (by means of isolating and evaluating the separated compounds) have not been reported, although some correlations have been made between total anthocyanins and perceived astringency. In general, the anthocyanin content increases during ripening of the berry and, simultaneously, the astringency decreases. The contents of tannins and some other phenolic compounds may decrease at the same time. The berries in these studies had a very intense colour and it would be interesting to isolate anthocyanins from the other phenolic compounds and study their orosensory characteristics. However, the fraction needs to be carefully purified from the other phenolic and non-phenolic components. Additionally, extracts with intense colour due to the anthocyanins may dye intensely. Thus, overly high contents may be negative factors for consumers.

The press residues were not that astringent or bitter compared to the extracts, regardless of the highest contents of these compounds in the skin fractions of the berries. The compounds contributing to astringency may be released from the skin structures more effectively with ethanol than with human saliva. The compounds may be bound to the fibre fractions of the skin-rich residue and not be as biologically available in the intestine as they may be after separation. First isolating the phenolic compounds and then adding them back to the juice fraction may be used to enhance the contents of these compounds. Compounds may be added to some extent without significantly altering the sensory characteristics of the juice. Although the addition experiments were not done with BC, the increase may be higher with this method than by using enzymes. However, in both cases, extraction and enzymatic treatments, the astringency increased along with content of phenolic compounds and thus the process must be carefully adjusted for each berry and/or food product.

6.2 Enzymatic treatments

The use of enzymes significantly increased the yields of the BC juices. Hydrolysis of berry cell walls and carbohydrates was achieved using a mixture of pectinolytic, cellulolytic and hemicellulolytic enzymes. Only commercially available enzymes, which are known to possess various activities, were chosen for this study. These were same enzymes available for applications in the food industry.

Although the differences between juice samples in study IV were relatively small, excluding the difference between enzymes and no enzymes, some notable and significant trends were detected in the analyses which are combined in Figure 16. At the same time as the overall yield of the juice increased, the intensities of astringency and fermented odour and flavour increased in the juices prepared with enzymes. These sensory properties may be regarded as negative factors. The use of pectinolytic enzymes in preparing the juices increased the astringency of the samples. Pectins may bind polyphenols and thereby lower the perceived
astringency (Bajec and Pickering, 2008). As pectinases degraded the pectin in the juices, they may have also increased astringency at the same time.

The heat treatment alone had some effects on the BC juices as the fresh odour decreased, but the contents of various phenolic compounds increased. Some pasteurisation is required for berry juices to inhibit the growth of moulds and yeasts. A higher incubation temperature resulted in higher pectinase activity and thus resulted in increases of various compounds. Therefore, more efficient degradation of pectin and improved solubility of sugars and bioactive compounds were achieved. Nevertheless, minimal processing and thus lower incubation temperatures are generally preferred in the food industry. The juices prepared using an additional enzyme with high glucosidase activity (Macer) had higher contents of some phenolic compounds than the other juices prepared using only the Biopectinase. In addition to a slight increase in anthocyanins, the use of Macer increased the flavonol aglycones and degradation products of hydroxycinnamic acid derivatives. In general, more processing (higher incubation temperature or more enzymes) resulted in increases in various phenolic compounds, but simultaneously increased astringency, fermented odour and flavour and/or sourness.

Enzymatic treatments in juice processing made the juices significantly less viscous resulting in similar yields to the crowberries and bilberries. The two other berries had very low viscosity and with these berries, enzymes may not be needed to increase the yield. The contents of various phenolic compounds may be significantly increased by adding extracts (made from the press residues) to their juices. Similar additions may not be as easy to conduct with BC juices. The high pectin content of BC may mask the astringency of the added phenolic compounds. Nevertheless, it is still very reasonable to use enzymes for juice production with various plant materials by carefully controlling and optimising the process conditions during juice making with a view to ensuring adequate sensory qualities of the juice.

Storage for zero to six weeks had little impact on the sensory profiles of the BC juices. Only an increase in sourness was detected in juices prepared with a mixture of pectinolytic, cellulolytic and hemicellulolytic enzymes compared to juice prepared only with a pectinolytic enzyme. In both cases, the losses of anthocyanins and ascorbic acid were significant along with losses or degradation of some other components. A storage period longer than six weeks may have resulted in more notable differences in the juices. In these samples, however, we used potassium sorbate, which is a common food preservative to inhibit the growth of moulds and yeasts (Belitz et al., 2004).
Figure 16. Summary of significant trends in study IV

Heat treatment vs untreated
- no impact on the yield
- no impact on sugar/acid ratio
- ↑ various phenolic compounds
- ↓ fresh odour, sucrose

Untreated juice

Enzymatic treatment vs untreated
- significant increase in the yield
- ↑ various phenolic compounds, fruit acids, ascorbic acid, sugars
- ↑ fermented odour and flavour, astringency, sourness, bitterness
- ↓ fresh odour, sweetness, roundness, sucrose, sugar/acid ratio

Heat treatment
- Incubation
  - 40 °C vs 50 °C
  - ↑ sourness
  - ↑ total sugars, glucose and fructose
  - ↑ total fruit acids, citric, malic and quinic acids
  - ↑ total phenolics and total anthocyanins

- Pasteurisation
  - 80 °C vs 90 °C
  - No impact

Enzymatic treatment
- Biopectinase + Macer
  - Increasing dosage of Macer
  - ↑ yield
  - ↑ astringency
  - ↑ fermented odour and flavour
  - ↑ flavonol algycons and degradation products of hydroxycinnamic acid conjugates
  - ↑ total phenolics and total anthocyanins
  - ↓ hydroxycinnamic acid conjugates

Storage
- Only Biopectinase
  - 0 to 6 weeks
  - ↑ some flavonol glycosides and their aglycons
  - ↑ degradation products of hydroxycinnamic acid conjugates
  - ↓ anthocyanins
  - ↓ sucrose
  - ↓ malic acid
  - ↓ ascorbic acid
  - ↓ hydroxycinnamic acid conjugates

- Mixture of enzymes
  - 0 to 6 weeks
  - ↑ yield
  - ↑ sourness
  - ↑ some flavonol glycosides and their aglycons
  - ↑ degradation products of hydroxycinnamic acid conjugates
  - ↓ anthocyanins
  - ↓ all sugars
  - ↓ total acids, malic acid
  - ↓ ascorbic acid
  - ↓ hydroxycinnamic acid conjugates
6.3 Berry liking and individual differences

Because the interactions between sensory properties and chemical compositions were investigated in studies I–IV, their roles in liking of berry fractions juices was investigated in study V. Especially, the impact of adding extracts to the juice fractions on sensory properties and thus liking was investigated. The juices and juices supplemented with extracts were food models rather than extracts. Therefore, the extracts were left out from the hedonic test, but they were included in the sensory evaluations. In study III, the addition of extracts to the BB juice did not alter the sensory profile of the juice. However, a small but not statistically significant increase was detected in astringency using the linear scale. In a later study (V) using the gLMS scale, similar effects were detected with BB juice. The addition did not affect the soft, velvety astringency, which was the more abundant of the two astringent subqualities, but increased the rough astringency. However, the liking of the BB juices was not affected. In the case of CB juices, the addition resulted in increases in bitterness and rough astringency and thus a decrease in liking. Particularly, bitterness and various astringent properties in foods with a high content of phenolic compounds may have adverse effects on consumer acceptance (Lesschaeve and Noble, 2005). All in all, the contents of various phenolic compounds, which are generally considered healthy, may be increased without altering the sensory profile or liking, but the amount to be added needs to be carefully adjusted for each berry material. Increasing the amount of phenolic compounds in foods by adding extracts may have negative effect on consumer acceptance (Jaeger et al., 2009). Although the health promoting properties and functionality of foods may be increased by supplementing with extracts, consumers may still not be willing to compromise on the taste for increased health benefits (Verbeke, 2006).

In general, sourness can evoke rejection responses in humans, but may appear attractive at low concentrations (Kim et al., 2004). A certain level of sourness may thus be expected by consumers when consuming berries or berry products. Sweetness, on the other hand, is a generally liked attribute, at least to some extent. Sucrose may be used to mask bitter and astringent properties of polyphenol-rich foods (Ares et al., 2009). However, the addition of sucrose may increase acceptance or decrease the aforementioned characteristics only to some extent (Jaeger et al., 2009). On the other hand, the nutritional quality of berry products would be higher without extra sugar addition. Both CB and BB juices had low contents of sucrose and other sugars were more abundant. Nevertheless, the sucrose content was significantly higher in the BC juice (prepared without enzymes). The sugars and organic fruit acids originating in the berry juice fractions may mask the negative sensory features (bitterness and astringent properties) contributed by the phenolic compounds. The difference between the abundance of sugar and acid compounds between the berries may, in part, affect this masking effect and thus liking. Some astringent subqualities in the berries may be linked to sourness and organic acids and low pH. In study V, sourness was a positive driver of liking and it did not correlate with either of the two astringent properties (soft or rough). These astringent attributes may be the more disliked features of the berries rather
than the actual sourness. CB juices as well as BB juices may have lacked the astringent subqualities of non-phenolic organic acids as compared to the BC juices, which had the attribute of sharpness in addition to sourness.

The two astringent references in study V were perceived notably different at the concentrations they were presented. This difference was confirmed from every subject. Both references had mouth-drying properties, but only AlSO₄ had roughing properties whereas these were not found in NH₄Al(SO₄)₂, thus making it softer. The subjects were asked to focus on the sub-qualities instead of the whole astringent sensations. The terms "soft" and "rough" may sound as the opposite ends of a scale, but the velvety and puckering (respectively) were used with those terms, as well as many other helping descriptions.

The subjects in the study V were chosen according to the prescreened hTAS2R38 genotype. The results showed that the genotype groups were different in perceiving berry samples. The AVI/AVI group rated bitterness, sourness and astringent properties of berry fractions higher than the sensitive PAV/PAV subjects, unlike originally expected. Due to choosing only the CB and BB juices for the hedonic test, the liking of the extracts was predicted using PLS regression models. The less intense sensory profile of the PAV/PAV resulted in notably lower predicted liking values for the sensitive subjects than the profile of the AVI/AVI. For the PAV/PAV individuals, both the soft, velvety and the rough, puckering astringencies were more negative drivers for liking in the PLS regression models; for the AVI/AVI subjects, the former property was more positive driver and the latter less negative. Genotype grouping may affect the overall profile of food as well as other orosensory properties. It is thus important to discriminate the various astringent subqualities as they may have different contributions to the sensory profiles and to the acceptance of astringent foods. The results of these predictions supported previous findings (Pickering and Robert, 2006) that sensitive bitter tasters were different from insensitive tasters in discriminating and rating various astringent subqualities.

The variation in the hTAS2R38 genotype may have an impact on dietary habits and furthermore on eating behaviour. Duffy et al. (2010) reported that sensitive individuals consumed fewer vegetables than insensitive subjects regardless of the bitter properties of the vegetables. Individual differences based on the hTAS2R38 gene in bitter perception of food samples have been connected often to glucosinolate producing vegetables such as Brassicaceae family species (Sandell and Breslin, 2006). Although glucosinolates have not been reported in berries, all food compounds, which are the key molecular activators of the TAS2R38 taste receptor or other bitter receptors, are not yet known. Bignay (Antidesma bunius) is an example of berry showing the connection between food taste perception and hTAS2R38 (Henkin and Gillis, 1977; Tharp et al., 2005). The PTC-sensitive subjects (PAV/PAV) rated bignay berry as sweet, while the PTC-insensitive (AVI/AVI) subjects rated and described the same berry as bitter. These previous findings and the results of study V show that the hTAS2R38 genotype may affect the individual orosensory profiles of berries and
berry products. Additionally, the “Mood” subscale was the only motive subscale which correlated directly with subject genotype grouping. Mood was more important to the PAV/PAV subjects than to the AVI/AVI subjects.

The two CB juices were less liked than the BB juices. At the same time, the CB juices were liked more by the subjects who were more concerned about their health and stated greater importance for health, weight control and the natural content of food. Simultaneously, the older subjects were more concerned with their health and stated greater importance in the same food choice motives. Health drinks, dairy products and rye breads have been reported to be more pleasant for those people stating healthiness as an important motivation in the food selection, compared to people preferring other motivation factors (Pohjanheimo and Sandell, 2009a; Pohjanheimo and Sandell, 2009b, Pohjanheimo et al., 2010). The health related motives and concerns may have strong impacts on berry liking with some individuals and thus may increase the consumption of berries, regardless the possible “negative” sensory properties of the berries.

Various other sensory attributes besides taste and astringency, may affect the liking, as well, but they were not studied here. Especially, the aroma components in juices and extracts may have influenced the liking ratings. The odour and appearance had strong influences on overall liking. Bilberries (along with blueberries) can be referred to as well-recognised berries. Their odour was more liked in our study than the odour of the less familiar crowberry. The subjects who stated a higher importance in the familiarity rated the odour of bilberry juice as more pleasant. Although the odours of CB and BB juices were generally mild, the familiarity of odours has a notable role in berry liking. Black currants are also regarded as well-known berries and their odour may have a strong influence on liking. A loss of fresh odour and an increase in fermented odour with enzymatic processing may have notable influences on the liking of BC juices. Liking the appearance of CB juices correlated strongly with high health concerns and health importance. Thus, the subjects in the high groups on these subscales may have preferred deep blue coloured berries as the high anthocyanin contents may have been regarded as healthy.

The berries, berry fractions and other berry products with bitter and astringent properties may be liked and preferred by individuals who are concerned about their health and whose food choice motives include healthiness and weight control. In this study, the higher liking ratings made by those subjects did not correlate with more frequent usage. The orosensory attributes which contributed negatively to liking in berries may be linked to healthy properties, but do not necessarily encourage consuming more berries. The high consumption rate of berries or berry products by the subjects in the study is most likely due to the recruiting protocol.
6.4 Methodological considerations

The analyses and identifications of the phenolic compounds were conducted using a relatively simple method without numerous separation steps. This resulted in overlapping peaks in HPLC chromatograms and difficulties in quantification. More efficient separation methods (additional extraction methods, column chromatography or preparative HPLC) may have resulted in better quality in identification and quantification. Additionally, analysing the complete chemical structures, including isomeric structures, of the phenolic compounds (e.g. with NMR) would have given more precise information on how the compounds may taste and interact. Commercial reference compounds were available for only a few of the identified compounds. Therefore, many of the compounds were quantified using the other compounds available, which may have influenced the results. In studies I–IV, only some of the possible sensory-active components were analysed. Additionally, these analysed components were shown to be sensory-active in other studies rather than in these studies. The compounds were not first isolated to confirm their own sensory properties.

In studies I–IV, only one-way ANOVA results were reported. However, a mixed three-way ANOVA, where samples are fixed factors and session and assessors are random factors, would have given more informative results concerning the panel performance and sample differences. Some three-way ANOVAs were used in studies I–IV, but because primarily the main effects were only significant, they showed same results as one-way ANOVAs. Additionally, in these previous ANOVA models, all factors were treated as fixed rather than treating assessors and sessions as random. For the study V only, the mixed three-way ANOVA was used.

The liking of the extracts, especially due to the predicted difference between subject genotype groups, needs to be further verified. Because of by highlighting the impact of adding extracts to the juices, the extracts were left out from the hedonic test. The differences between genotype groups were thus shown only with the predicted liking ratings. Although the results showed that the grouping based on hTAS2R38 genotype may influence the liking of certain polyphenol-rich fractions of berries, the extracts may only be considered as potential food ingredients rather than foods. The differences in hTAS2R38 genotype with regards to berry perception and liking also need to be studied with other berries and berry materials. Further, the hTAS2R38 genotype is only one indicator of the phenotype, which may be affected by various other genes and factors. Therefore grouping based on this genotype is somewhat different than the PTC- or PROP-status. The subjects in study V were recruited to a berry study and thus they may have been familiar with berries rather than disliking them. The frequency of berry consumption was asked in general without asking the details of consumption. Further studies are needed to link the individual differences and the use of berries in different forms.

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7 CONCLUSIONS

The results of this thesis provide a new understanding on the different astringent properties of various food components, especially in plant materials. The results were achieved by combining theory and methods of sensory food science, food technology, analytical chemistry, statistics, food psychogenomics and consumer behaviour research using berries as the target material.

The results showed that blue coloured berries have significantly different orosensory profiles. Due to different compositions, berries may be different in terms of sourness, sweetness, bitterness and may have different astringent subqualities. In general, sourness and sweetness are located in the juices of berries and the bitterness and astringency of the phenolic compounds is located in the skin fraction of the berries. Additionally, the use of enzymes increased the yield of many potentially health-beneficial berry components in juice processing, but simultaneously increased astringency and fermented odour and flavour. Maximising the yields from berry raw materials may contribute to negative sensory characteristics and needs careful optimisation. Although some enzyme treatments may produce less optimal flavour properties, they can be used to obtain new flavours and properties as well. The taste properties of berries may be affected or modified by processing the berries, as the concentrations of the key compounds varies in different parts of the berries and some of these compounds may change or be released during processing. Berries behave differently in processing, because of their own unique flavours and chemical profiles. Thus different special methods (e.g. ethanol extraction or enzyme treatment) may be used for producing various special food products.

Sourness of berries is a positive factor in liking and it may be liked to some extent. However, the astringent subqualities related sourness may have aversive effects. Different astringent subqualities of phenolic compounds may also have different contributions to liking of berries. Rough and puckering astringency is more disliked than soft and velvety astringency. Nevertheless, there are various factors influencing individual berry perception and liking. These include various behavioural as well as genetic factors.

Berries can be regarded as healthy foods and they should be part of a healthy diet, but the negative orosensory characteristics may have a negative influence on their acceptance and further consumption. The multidisciplinary approach of this research may be useful when exploiting berries for food development. All parts of berries can be used more effectively in the food industry when the key sensory characteristics and the compounds contributing to these attributes are known.
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