Quality of fruits and purees of strawberries as affected by genotype, maturity and processing

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Abstract

Strawberries (*Fragaria X ananassa*) contain a wide range of nutrients and bioactive compounds. In this thesis the main focus was on anthocyanins (red pigments), activity of polyphenol oxidase (PPO), anthocyanin degrading enzyme, and colour of strawberries. The aims of the study were 1) to determine quality and chemical composition of strawberry fruits from different genotypes and maturity, and 2) to find the effects of strawberry traits (genotype and maturity) and treatments (temperature treatment and storage time) on the content of total monomeric anthocyanins (TMA), activity of PPO and colour stability of strawberry purees.

Eight genotypes of strawberry, two of them with three degree of ripening (Polka and Senga Sengana), were investigated for their chemical composition (dry matter, pH, °Brix and TMA), the colour parameters (L* °Hue, Chroma and acceptance factor) and the activity of PPO. Three cultivars (Polka, Senga Sengana and Sonata) were further studied for PPO activity, TMA content and colour parameters as affected by processing, that is thermal treatment (85 °C for 5 minutes) and storage (22 °C for 0, 2 and 5 weeks). Three degrees of Polka maturation were included in the analysis and only medium maturation stages of the other two, Senga Sengana and Sonata. PPO was analysed with the spectrophotometric method, TMA with the pH-differential method and colour parameters with the CIELAB colour system.

The findings revealed that genotype and maturity states affected chemical composition of the berries. Storage induced profound degradation of anthocyanins and colour change in the puree. Heat treatment had no significant effect on levels of TMA and it only partially inactivated PPO. Undesired effect of the thermal treatment was that it had a negative effect on colour of strawberry purees.

Sammendrag

Jordbær (Fragaria X ananassa) inneholder et bredt spekter av næringsstoffer og bioaktive forbindelser. I denne avhandlingen var hovedfokuset på antocyaniner (røde pigmenter), aktivitet av polyfenoler oksidase (PPO) (et enzym) og fargen på jordbær. Målet med studien var 1) å bestemme kvalitet og kjemisk sammensetning i fruktene av jordbær fra ulike genotyper og modningsgrad, og 2) å finne effekten av jordbærtyper (genotype og modenhet) og faktorer (varmebehandling behandling og lagringstid) inneholder av totalt monomere antocyaniner (TMA), aktivitet av PPO og farge stabilitet i jordbærpuré.

Åtte genotyper av jordbær, to av dem med tre modningsgrader (Polka og Senga Sengana) ble undersøkt for deres kjemiske sammensetning (tørrstoff, pH, ° Brix og TMA), fargeparametere (L * ° Hue, Chroma og aksept faktor) og aktiviteten av PPO.

Tre genotyper, og en med tre modningsgrader (Polka), ble videre undersøkt for PPO aktivitet, TMA-innhold og fargeparameterne som kan bli påvirkes av prosessering, som er varmebehandling (85 °C i 5 minutter) og lagring (22 °C i 0, 2 og 5 uker) PPO ble analysert med spektrofotometer-metoden, TMA med pH-differensial metoden og farge parametere med CIELAB fargesystem.

Resultatene viste at genotype og modningsgrad induserte forskjeller i den kjemiske sammensetning av bærene. Varmebehandling hadde ingen signifikant effekt på nivåene av TMA og det bare delvis inaktiverte PPO. Uventet, ble termisk behandling funnet å ha en negativ effekt på fargen under lagring av jordbærpuré.

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1 Introduction

Colour is an important quality trait for berries and jam. The components that give colour to strawberries are anthocyanins, which are polyphenols that belong to the larger group of flavonoids. In addition to being responsible for colouring, these components also exhibit antioxidative properties that can have health benefits

When berries are processed (e.g. jam production) the cell structure in the berries is damaged, and the substances like anthocyanins are subjected to enzymatic and non-enzymatic degradation. The degradation continues during the storage period especially if stored at room temperature, and eventually leads to discoloration of the product. The colour change is a direct consequence of the degradation of anthocyanins. The colour is also affected by formation of brownish polymeric compounds formed after oxidation of anthocyanins and other phenolic compounds.

Fruits and vegetables contain degrading enzymes, such as peroxidase and polyphenol oxidase (PPO). If these enzymes are not inactivated during processing, they will catalyse enzymatic oxidation of anthocyanins and other phenolic compounds in the products during storage. In strawberries, PPO is the main degrading enzyme.

The goal of this thesis was to examine the PPO activity, pH and colour, as well as the content of total monomeric anthocyanins (TMA), brix and dry matter in eight different cultivated strawberry genotypes, two with three different maturity states (Polka and Senga Sengana) An additional goal was to assess the effect of heat treatment, storage time on selected strawberry types and strawberry cultivars on TMA, activity of PPO and colour.

2 Strawberries: theoretical background

2.1 Botanical and general characteristics

Wild strawberry (Fragaria virginiana) and cultivated strawberry (Fragaria X ananassa) are plants in the Rosaceae family. The fruit of the strawberry plant is composed of several small fruits that together make the whole fruit, where each small fruit has one seed called achene. Different tissues of the berry are illustrated in Fig. 1. Strawberry is known for its delicate flavour and rich vitamin content. Strawberry is found in all arable regionsm while the most of the production is located in the northern hemisphere (98 %). However, there are no genetic or climatic barriers preventing expansion to the southern hemisphere (Jules and Paull 2008). The fruits are consumed both as fresh and processed. In 2009, more than 4.1 million tons of strawberries were produced worldwide.

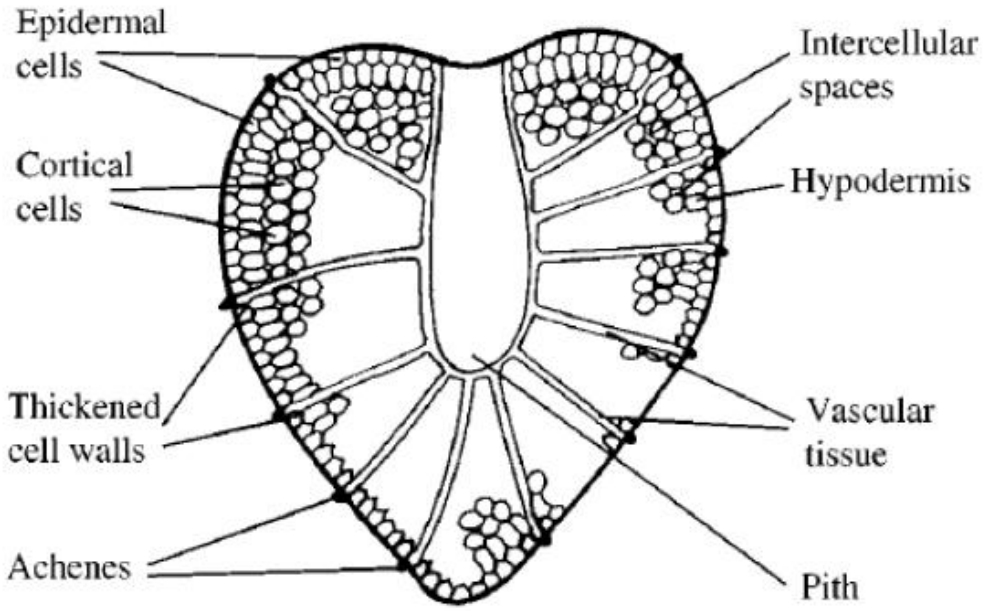

Fig 1: Strawberry structure. Outer layer is made up of epidermal cells while the internal parts are made up of hypodermal and cortical cells. Taken from: (Jewell et al. 1973).

Numerous epidemiological studies have shown that high intake of fruits and vegetables protect against several chronic diseases (Joshipura et al. 2001, Anttonen et al. 2006). The nutrient profile of strawberries, (especially high content of micronutrients) makes them a healthy food choice.

2.2 Effects of maturity state and genotype

Fruits become more colourful, sweeter and softer during maturation. But strawberry is a non-climacteric fruit, meaning that the fruit will not continue maturation after harvesting (Kader 2002). And thus, the composition of the fruit is highly dependent on when the fruit is harvested. For strawberries it takes about 30 days to become fully ripened, but this time frame is highly dependent on light, temperature, soil composition, and other condition of cultivars (Cordenunsi et al. 2002). Furthermore, different genotype also affects composition of the berry. For example, Cordenunsi et al. (2002) reported that sugar, acid and phenolic compounds varied in different genotypes of strawberry. Acidity and sugar levels are two main factors that determine taste of fruits which is also considered as quality parameter for strawberry. Furthermore, volatiles (such as ester, aldehydes, acids, alcohol, ketons and esters) contribute to the taste of fruits (Kader 2002). Moreover levels of water content can contribute to the firmness of strawberry, due to the cell pressure which can affect mechanical resistance of the tissue. However, Cordenunsi et al. (2002) reported that water content is quite similar for different strawberry types.

2.3 Chemical composition

Matured strawberries contain approximately 10 % total soluble solids and 90 % water. Strawberry is known for having a large diversity of dietary components and especially for its high value of vitamin C. The total sugar content is 40 % of the dry matter and consists mainly of glucose and fructose. Citric acid is a primary acid in strawberry, but also considerable amounts of ellagic acids have been reported (Jules and Paull 2008). For the full overview of nutrients in strawberry, see Table 1. In addition to this, strawberries contain a variety of different phenolic compounds (flavonoids, hydrolyzable tannins and phenolic acids) (Giampieri et al. 2012). These are not considered as classical nutrients but have many health promoting effects.

Table 1 list of nutrinens in strawberry (Giampieri et al. 2012)

Type	Nutrient	Per 100 g
Proximates	Water (g)	90.95
	Energy (kcal)	32
	Protein (g)	0.67
	Ash (g)	0.40
	Total lipid (g)	0.30
	Carbohydrate (g)	7.68
	Dietary fiber (g)	2.0
	Sugars (g)	4.89
	Sucrose (g)	0.47
	Glucose (g)	1.99
	Fructose (g)	2.44
Minerals	Calcium (mg)	16
	Iron (mg)	0.41
	Magnesium (mg)	13
	Phosphorus (mg)	24
	Potassium (mg)	153
	Sodium (mg)	1
	Zinc (mg)	0.14
	Copper (mg)	0.048
	Manganese (mg)	0.386
	Selenium (µg)	0.4
Vitamins	Vitamin C (mg)	58.8
	Thiamin (mg)	0.024
	Riboflavin (mg)	0.022
	Niacin (mg)	0.386
	Pantothenic acid (mg)	0.125
	Vitamin B6 (mg)	0.047
	Folate (µg)	24
	Choline (mg)	5.7
	Betaine (mg)	0.2
	Vitamin B12 (µg)	0
	Vitamin A, RAE (µg)	1
	Lutein + zeaxanthin (µg)	26
	Vitamin E, α-tocopherol (mg)	0.29
	β-tocopherol (mg)	0.01
	γ-tocopherol (mg)	0.08
	δ-tocopherol (mg)	0.01
	Vitamin K, phylloquinone (µg)	2.2

2.3.1 Phenolic compounds

Phenolic compounds represent a large and heterogeneous group with many different families of secondary metabolites (Alonzo-Macías et al. 2013) and can be classified into flavonoids and non-flavonoids (Crozier et al. 2006). They are characterized by at least having one aromatic ring. More than 8000 different structures of phenolic compounds have been reported in the plant kingdom (Crozier et al. 2006). Earlier it was believed that the health promoting effects of strawberries are mainly due to the antioxidative activities of phenolic compounds. Recent studies reported their important roles (Battino et al. 2009). For example, it was shown that phenols are involved in modulation of cellular signalling pathways that can also result in changes of gene expression. Phenolic

compounds were also shown to possess antimicrobial, antiallergic, and antihypertensive properties (Battino et al. 2009). The major class of phenolic compounds in strawberries are flavonoids. For the full overview of phenolic compounds in strawberries, see Table 2.

Table 2 Reported phenols in strawberry (Giampieri, et al 2012)

Class	Group	Compound
Flavonoids	Anthocyanins	Cyanidin-3-glucoside
		Cyanidin-3-rutinoside
		Cyanidin-3-malonylglucoside
		Cyanidin-3-malonylglucosyl-5-glucoside
		Pelargonidin-3-galactoside
		Pelargonidin-3-glucoside
		Pelargonidin-3-rutinoside
		Pelargonidin-3-arabinoside
		Pelargonidin-3,5-diglucoside
		Pelargonidin-3-malylglucoside
		Pelargonidin-3-malonylglucoside
		Pelargonidin-3-acetylglucoside
		Pelargonidin-dissacharide (hexose + pentose) acylated with acetic acid
		5-pyranopelargonidin-3-glucoside
	Flavonols	Quercetin-3-glucuronide
		Quercetin-3-malonyglucoside
		Quercetin-rutinoside
		Quercetin-glucoside
		Quercetin-glucuronide
		Kaempferol-3-glucoside
		Kaempferol-3-malonyglucoside
		Kaempferol-coumaroyl-glucoside
		Kaempferol-glucunoride
	Flavanols	Proanthocyanidin B1 (EC-4,8-C)
		Proanthocyanidin trimer (EC-4,8-EC-4,8-C)
		Proanthocyanidin B3 (C-4,8-C)
		(+)-catechin
Phenolic acids	Hydroxycinnamic acids	*p*-coumaroyl hexose
Hydrolyzable tannins	Ellagitannins	Ellagitannin
		Bis-HHDP-glucose
		Galloyl-HHDP-glucose
		HHDP-galloyl-glucose
		Galloyl-bis-HHDP-glucose
		Dimer of galloyl-bis-HHDP
		Sanguiin H-6
		Methyl-EA-pentose conjugates
		Ellagic acid pentoside
		Ellagic acid

2.3.1.1 Flavonoids

Flavonoids consist of fifteen carbons, with two aromatic rings and a tree carbon ring (Crozier et al. 2006). Anthocyanins are the major class of flavonoids in strawberries, whereas flavanols and flavonols are found in smaller amounts (Giampieri et al. 2012). These categories are involved in many processes in plants, e.g. plant growth and protection against infection. The flavonoids are of particular interest, because of their multiple roles in plants and its impact on human health (Harborne and Williams 2000). In the class of flavonoids, a large group of anthocyanins is present in strawberries. Anthocyanins are the main compounds that are responsible for the red-blue colour of many fruits and vegetables and directly contribute to the colour of fruits (Alonzo-Macías, Cardador-Martínez et al. 2013).

2.3.1.1.1 Anthocyanins

Anthocyanins are water soluble compounds with the basic structure of the flavylium cation with the addition of a sugar group (He et al. 2012)(Fig. 2a). The anthocyanins that have been found in strawberries are glucoside and malonylglucoside of pelargonidin and cyaniding, and rutinoside and acetylglucoside of pelargonidin (Aaby et al. 2012). Furthermore, the report found pelargonidin-3-glucoside to be the most abundant anthocyanin in strawberry (60-95 %) and pelargonidin-3-malonyglucoside as the second highest (0-33.5 %). Cyaniding 3-glucoside and pelargonidin 3-rutinoside have also been reported in smaller amounts (Aaby et al. 2007). For full list of anthocyanins found in strawberries, see Table 2.

Anthocyanins are also shown to have great health promoting effects. Epidemiologic studies have shown that they lower the risk of cardiovascular and neurologic diseases, infections, obesity and cancer. Different *in vitro* oxidation studies have shown that the antioxidant activity in fruit is directly correlated with the anthocyanins content (Alonzo-Macías et al. 2013).

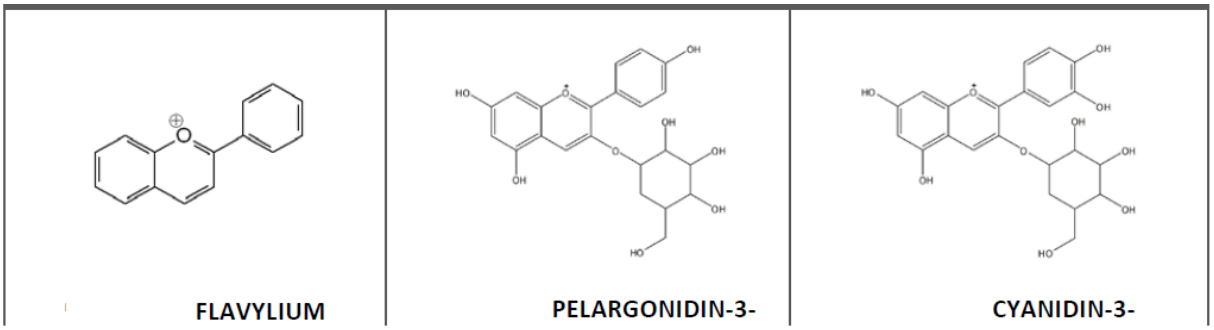

Fig. 2. Basic structure of the flavylium, and the two structures of anthocyanins that give colour to strawberry. Taken from: (Avasoo and Johansson 2011)

2.3.1.1.2 Colour compounds

Anthocyanins are the main phenolic compounds that give strawberries their red colour. Pelargonidin 3-glucoside and cyaniding 3-glucoside, illustrated in figure 2b and 2c, are the anthocyanin pigments that are responsible for the bright red colour in strawberry (Jules and Paull 2008, Ngo et al. 2007). Anthocyanins can also create complexes with other coloured products and give discolouring or coloured effects (Gössinger et al. 2009a). The degradations of phenolic compounds to polymers have a direct consequence on colour change of the fruit. (Dalmadi et al. 2006). When anthocyanins are degraded, by e.g. heat, they form smaller phenolic compounds, anthocyanidins and sugar(Sadilova et al. 2007) . The anthocyanidins can be further broken down to even smaller phenols that can be oxidized by enzymes to form brown pigments. Other phenolic compounds like cartechins and quercetin can also be subjected to enzymatic browning and contribute to discolouring of the product (Terefe et al. 2010). Degradation of phenolic compounds results in bleaching or browning of the product (Gössinger et al. 2009a).

2.3.1.1.3 Total monomeric anthocyanins (TMA)

Anthocyanins are commonly found in the monomeric form. Amount of monomeric anthocyanins can be measured spectrophotometrically because monomeric anthocyanins undergo a reversible transformation due to a change in pH (coloured oxonium forms at pH 1.0 and colourless hemiketal forms at pH 4.5, (illustrated in fig. 3)). Polymerized anthocyanins and other degradation products or interfering compounds are resistant to colour change with change in pH. Thus, only monomeric anthocyanins are measured with the pH-differential method.(Lee et al. 2005).

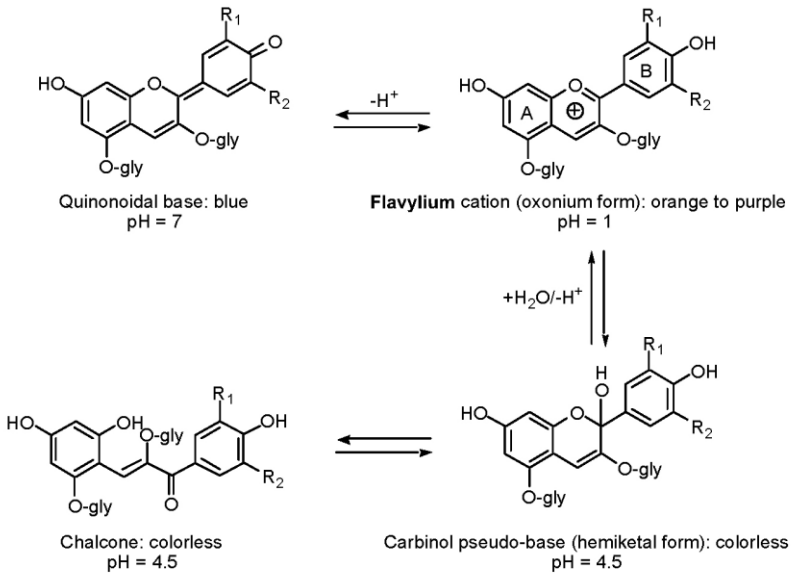

Fig. 3 Predominant anthocyanin structural forms. Taken from: (Avasoo and Johansson 2011)

2. 4 Effects of processing on colour compounds

Stability of phenolic compounds is affected by a number of different factors such as enzyme activity, temperature, acid content, pH and oxygen (Gössinger et al. 2009b). When berries are processed e.g. freezing, thawing and heat treatment, phenolic compounds like anthocyanins, that are highly unstable, become more accessible for enzymatic and non-enzymatic degradation. This is mainly due to the disruption of the cellular structure. This further leads to more rapid colour change and loss of nutrient value in the fruit (Chisari et al. 2007).

2.4.1 Non-enzymatic degradation

During the strawberry processing it is important to pay attention to several chemical reactions that can disrupt the final quality of the product. There are two common non-enzymatic degradation reactions that often occur in processed strawberries: the Millard reaction and the caramelisation (Garcia and Barrett 2002). Strawberries contain amino acids and sugars which are the substances needed for Millard reaction to occur. The reactive carbonyl group of sugar reacts with nucleophilic amino group of the amino acid and forms complex molecules that can easily react with other compounds to make brown polymeric pigments. However, this reaction

usually requires the product to be heated. Caramelisation, the browning of sugars, is also heat dependant. Both of these reactions occur during heat treatment but to a lesser extent during the storage (Sloan et al. 1969).

2.4.2 Enzymatic degradation

During the storage period two enzymes have been reported to have effect on anthocyanin degradation (Kader 2002). They are called polyphenol oxidase (PPO, EC 1.14.18.1) and peroxidase (POD, EC 1.11.1.7).

2.4.2.1 Polyphenol oxidase

PPO is mainly found in the chloroplast thylakoid membranes, and phenolic compounds mostly found in the vacuoles. When the tissue is damaged, the enzyme will come in contact with phenolic compounds and an oxidation reaction will start. This will finally lead to the browning of the fruit. The extent of browning depends on the total amount of phenolic compounds in the fruit and level of PPO activity (Kader 2002). PPO activity in strawberry is general around 1.307 OD/min/g (Terefe et al. 2010).

PPO is a copper containing enzyme involved in the synthesis of betalain and melanin which are responsible for the browning reaction (Chisari et al. 2007). It is found in a broad variety of bacteria fungi, plants, insects, crustaceans and mammals. It is activated by oxygen, and it catalyses various reactions such as monophenolase reaction cycle and diphenolase reaction cycle. In the monophenolase reaction, one of the bound oxygen atoms is transferred to a monophenol, generating o-diphenol intermediate, which is subsequently oxidized to o-quinone and released along with a water molecule (see fig. 4) (Kegg u/å). Dipheolase cycle the enzyme binds an external diphenols and oxidize it to an o-quinone that is released with a water molecule. Dipheolas reaction is identical to that catalysed by the related enzyme catechol oxidase (Kegg u/å). Furthermore, the activity of the enzyme is temperature dependent; in general enzyme activities are at its highest at temperature of around 50 °C and pH around 5.5. (Kader 2002). Inactivation of enzyme can be induced in several ways. Commonly used methods for enzyme inactivation include heat and pressure treatments, adding of enzyme inhibitors and acids (Dalmadi et al. 2006).

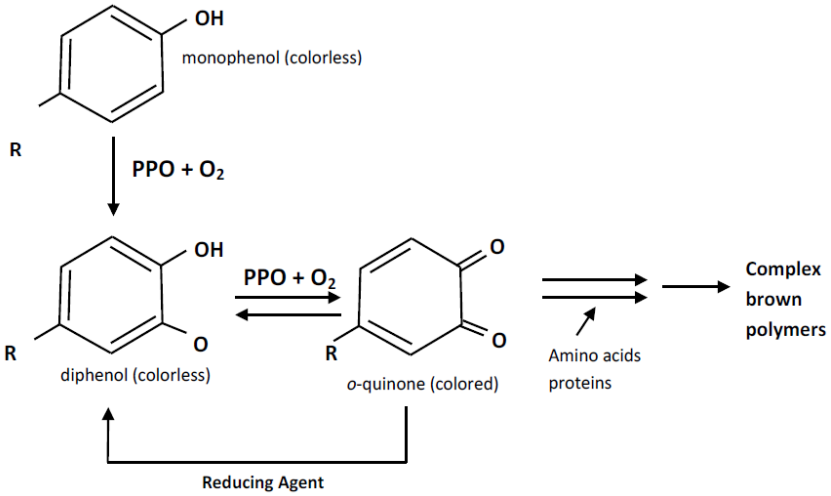

Fig 4. The catalysation of monophenol s to diphenols and eventually to o-quione, which may react to brown compounds. Taken from: (Avasoo and Johansson 2011)

2.4.2.2 Peroxidase

POD is another enzyme found in the vascular tissue of strawberries. It catalyses the phenolic compounds in the presence of hydrogen peroxide (López-Serrano and Ros Barceló 2002). Since the amount of present oxygen is higher than the levels of hydrogen peroxide and since POD is more thermo-labile than PPO, POD is considered to contribute little to the oxidation of phenolic compounds like anthocyanins (Terefe et al. 2010).

2.5 Colour as a quality parameter

Colour is a common quality parameter for costumers when purchasing foods. If the product doesn't have the "right" colour, a costumer may look at it as a spoiled product (Wrolstad et al. 2008). People also associate a certain taste with different colours of foods (e.g. red is associated whit sweet and yellow with sour). Therefore, changes in colour of the product are seen as potentially negative and should be avoided. Many studies have shown that the change in colour also gives a reduction of health benefits (Wrolstad et al. 2008). To measure colour in a sample, the CIE – system (Commission Internationale d`Eclariage) recommend CIELAB or CIE L*a*b* (Harold 1998) This system was developed to provide a standard for measuring colour (Norn 1997)

2.5.1 Colour measurements

The CIELAB colour system contains numbers of colour plates on top of each other. The wafer periphery comprises all colour tones. Colour intensity decreases towards the centre and the light strength decreases in lower region. (see Fig. 5)(Norn 1997) The CIELAB colour scale is an almost uniformed colour scale. In a uniform colour scale, the differences between points plotted in the colour space correspond to visual differences between the colours plotted (Lan 2007).

All colours are the combination of three colours; red, yellow and blue. The system is based on this. As CELAB colour spaces, represent the colours perceived by humans, and these are preferred for analysis in foods.

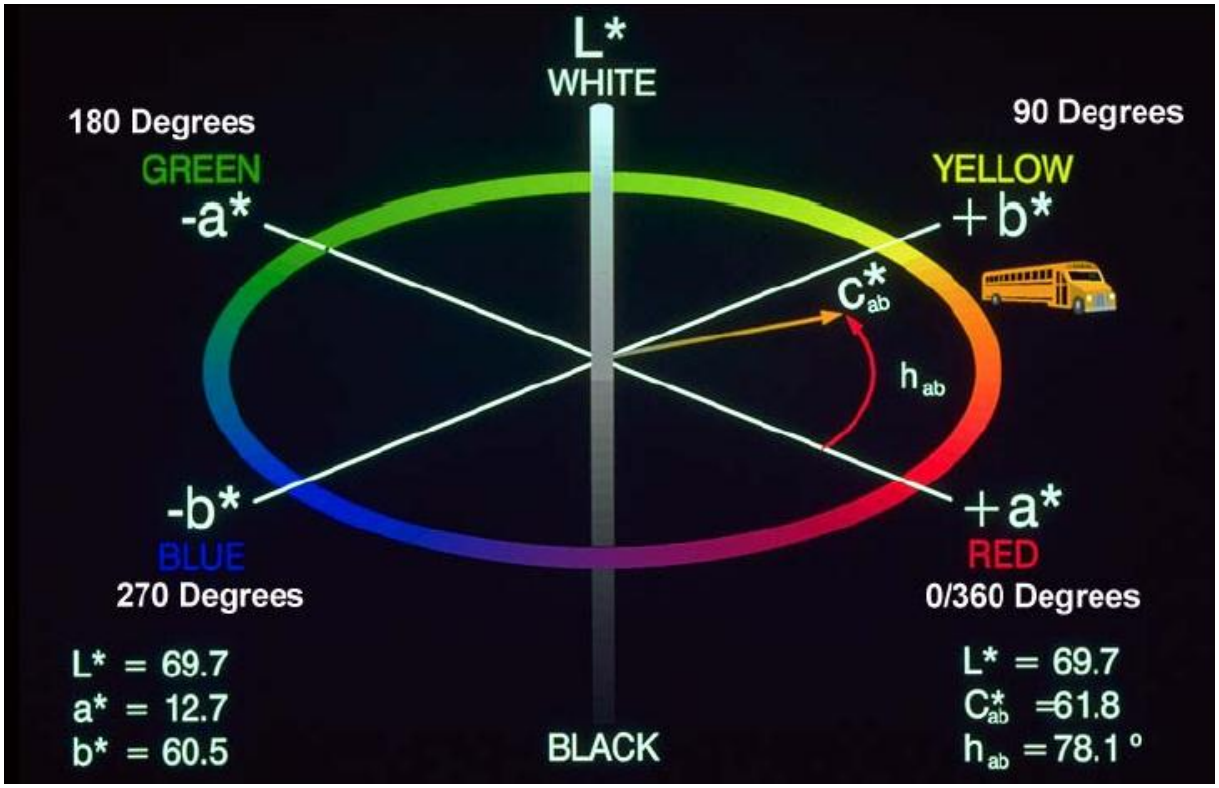

Fig. 5: *The L* axis runs from top to bottom. The maximum for L* is 100, which represents white. The minimum for L* is zero, which represents black. Positive a* is red; negative a* is green; positive b* is yellow; negative b* is blue; the a* and b* axes have no specific numerical limits.* Colour saturation = *Chroma (C^*_{ab}) and* colour tones = °Hue (h^*_{ab}) *is determined from these parameters. Taken from: (Harold 1998)*

Chroma is the length of the vector of colour point (see Fig 4). The higher the C°, the higher is colour intensity. °Hue indicates the hue of the colours, were °Hue of 0° or 360° represent red, yellow 90°, green 180° and blue 270° (see Fig. 6). Strawberries have °Hue values of 20-30 (Lan 2007).

Fig 6: The colours red, yellow, green and blue presented as degrees. Taken from: Wikipedia (u/å)

2.5.2 Acceptable factor (AF)

Acceptable factor (AF) is correlated with consumer acceptance of strawberry nectar. Gössinge 2009 at al. calculated AF from a survey with 600 costumers. The AF is calculated by dividing a*/°Hue (excellent [AF > 0.7], acceptable [AF > 0.4] or not acceptable [AF < 0.4])(Gössinger et al. 2009c)

3 Materials and methods

3.1 Materials

Eight different varieties of cultivated strawberries were used in this study and two of them with three different maturity states. The materials that were used were fruits of the varieties: Polka and Senga Sengana Florence, Blink, Sonata, GNKv 2078, GNKv 2031 and GNKv 2399, harvested from a field in the south – east of Norway (Apelsvoll) in 2011. The berries were frozen within 3 hours after harvesting and at -22 °C prior to analyses and processing.

3.2 Apparatus:

3.2.1 Spectrophotometry (*Shimadzu UV160U, Kyoto, Japan*)

3.2.2 Cuvettes

3.2.3 Pipettes

3.2.4 Volumetric flasks (1 l)

3.2.5 Flask (500 ml)

3.2.6 High-speed centrifugation tubes

3.2.7 50 mL plastic flask with screw cork

3.2.8 Beaker

3.2.9 Glass pipette

3.2.10 Food processor (Braun combiMax 700)

3.2.11 Centrifuge (*Becman Coulter Inc., Avanti^{R} J-36 XP Brea, California, USA*)

3.2.12pH- meter (*827 pH lan – Ω Metrohm USA Inc., Riveriew, Florida, USA*)

3.2.13 Homogenizer (*PT3300 polytron, Arlington Heights, Illinois, USA*) - (Knife: *Pt-DA-3012/3 WEC*)

3.2.14 Scale (Sartorius BP 310 s – (31 – 0.001)g) Ultra Turax)

3.2.15 Water bath (*Julabo Inc., SW22, Seelbach, Germany*)

3.2.16 Brix meter (*RE40, Mettler-Toledo Inc., Columbus, Ohio, USA*)

3.2.17 Hunter lab. Labscan XE with the source D_{65} spectrophotometer (*Hunter Associates laboratory, Inc., Reston, Virginia, USA*)

3.2.18 Vacuum oven (*W.C. Heraeus Hanau INc., RVT 360, Ridderkerk, Holland*)

3.2.19 Drying dish

3.2.20 Desiccator

3.2.21 Digital thermometer

3.2.22 Cylindrical measure cell with black rubber and lid

3.3 Chemicals

3.3.1 Catechol (110, 11 g/mol 1.2 dihydroxybenzene ($C_6H_6O_2$), CAS: 120-80-9, *Sigma-Aldrich, St. Louis, Missouri, USA*)

3.3.2 Triton X-100 (CAS: 9002-93-1, *Sigma-Aldrich, St. Louis, Missouri, USA*)

3.3.3 Natruimfosfat (*Merck KGaA, Darmstadt, Germany)*

a. Na_2HPO_4 (dibasic) (141.96 g/mol, CAS: 7558-79-4)

b. NaH_2PO_4 (monobasic) (137.99 g/mol, CAS: 89140-32-9)

3.3.4 1 M NaCl (*Sigma-Aldrich, St. Louis, Missouri, USA*)

3.3.5 Potassium chloride, KCl (74,56 g/mol , *Merck KGaA, Darmstadt, Germany)*

3.3.6 Sodium acetate, CH_3CO_2 NA•$3H_2O$ (82,03 g/mol, *Merck KGaA, Darmstadt, Germany)*

3.3.7 Sorbic acid (Hexa-2.4-dienoic acid ($C_6H_7O_2K$), CAS: 110-44-1, *Sigma-Aldrich, St. Louis, Missouri, USA*)

3.3.8 1 M HCl

3.4 Processing of strawberries

The experiment was performed in order to estimate the effect of heat and different storage time of 0 weeks, 2 weeks and 5 weeks on the amount of total monomeric anthocyanins (TMA), polyphenol oxidase (PPO) activity and the colouration of strawberries. The interaction between these three factors was also investigated.

3.4.1 Sample preparation

1400 g of strawberries was weighted (3.2.14) while frozen, and then thawed for one hour in room temperature, they were homogenized for 20 sec in a food processor (3.2.10), mixed with

1.4 g of sorbic acid (3.3.7) dissolved in 10 ml of water, and homogenised for another 20 sec. Strawberries were divided into 32 tubes (3.2.6), with 30 g in each. Next, 18 of the tubes were heat treated whereas the rest of the tubes did not undergo heat treatment. 2 replicates were made. The same procedure was done with 3 different varieties of strawberries (Polka with 3 different degrees of maturity and Sonata and Senga Sengana). A complete overview of the experimental design is presented in Fig. 7

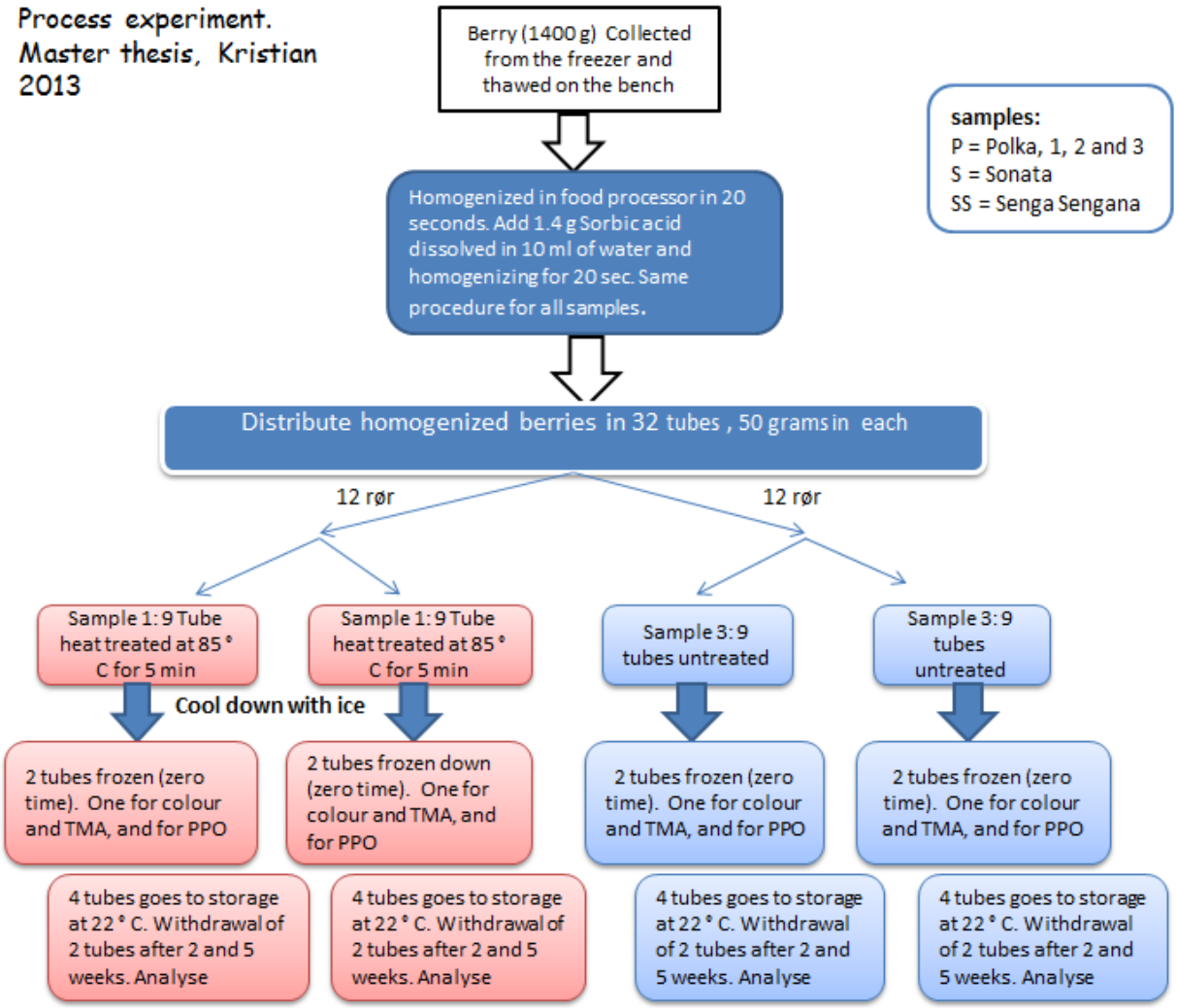

Figure 7: Overview of the experimental design

3.4.2 Heat treatment

The heat treatment was performed in a water bath (3.2.15). All samples had a starting temperature of 4°C. The samples were heated to 85°C and then kept for 5 min. After, the samples were immediately placed in ice cold water for rapid cooling.

Fig. 8: Water bath with the samples. Here one can see the thermometer in a test tube. Used a stop watch to measure the time.

Due to the long heating time for the samples (around 15 min), the water bath (3.2.15) was first set to 95°C, and then lowered to 86°C when the samples reached 81°C. To monitor the temperature in samples, an extra tube with the sample was immersed in the water bath in which the digital thermometer (3.2.21) was immersed (as presented in Fig 8). The desired temperature (85 °C ± 0.5) was reached in 15 min, followed by heat treatment for 5 min.

3.4.3 Storage

The heat treated and non-heat treated samples were stored for 0, 2 and 5 weeks before being frozen. They were stores in a dark room with a temperature of 22 °C

3.5 Sample Preparation for dry matter, pH, brix, total monomeric anthocyanins and polyphenol oxidase on the 8 different cultivars with three different maturity levels in Polka and Senga Sengana.

300 g of each strawberry were weight (3.2.14) up and thawed for ca. 20-30 min. Then they were homogenized in a food processor (3.2.10) before frozen down again as homogenized berries. The berries were never completely thawed. This homogenized berry was then used for analysing pH, brix, dry matter, TMA, PPO and colour measurements.

3.6 Analyses

3.6.1 Dry matter

The vacuum method was used for the estimation of dry matter. The samples were dried to constant weight under vacuum (≤ 50 mm Hg) at 70˚C. The samples were analysed in triplicates.

3.6.1.2 Sample preparation

Ten gram of homogenised berries (3.5) where evenly distributed over a drying dish (3.2.19). Then the dishes were placed in the oven (3.2.18) that was preheated to 70 (± 2) °C and dried for 2 h. Consecutively, the vacuum was applied in the oven for the duration of 24 h.

After the drying, the samples were transferred to a desiccator (3.2.20) and cooled down before weighting (3.2.14).

3.6.1.3 Calculations

Calculation of the dry matter was done as following:

$$\%\ dry\ matter = \frac{100 * (\mathrm{a} - \mathrm{b})}{(\mathrm{c} - \mathrm{b})}$$

Where:

a is the weight of drying dish with sample after drying,

b is the weight of the drying dish and

c is the weight of drying dish and sample before drying.

Dry matter was calculated as amount of dry matter per 100 g of wet sample (g/100 g).

3.6.2 Brix

Brix: Degrees Brix (symbol °Bx) present the sugar content of an aqueous solution. One degree Brix is 1 g of sucrose in 100 g of solution and represents the strength of the solution as percentage by weight (% w/w).

Homogenized strawberries (3.5) were used to measure the brix in a brix meter (3.2.16): first the instrument was calibrated with air, then with distilled water. Three replicates were taken.

3.6.3 pH

Fig. 9: pH meter used for measuring the samples

PH is a measure of the hydrogen ion concentration; value of 7 denotes a neutral, below 7 acidic and over 7 a basic solution. It is defined as the decimal logarithm of the reciprocal of the hydrogen ion activity, aH+, in a solution.

3.6.3.1 Procedure

The pH meter (3.2.12) (seen fig. 9) was calibrated with two solutions (solution 1 with pH 4.5, and 2 with pH 7.0). Samples were then measured by submerging the pH electrode in homogenised strawberries (3 replicates).

3.7 Polyphenol oxidase (PPO)

Method for measuring PPO activity in fruits and vegetables

3.7.1 Buffers

Two different buffers were made: (1) Na_2HPO_4 (dibasic sodium phosphate) (3.3.3a) and (2) NaH_2PO_4 (monobasic sodium phosphate) (3.3.3b). The dibasic solution was prepared in quantity of 1 liter, by adding 28,4 g/L of Na_2HPO_4 to a 1 liter volumetric flask (3.2.4) and mixing with approximately 0.85 liter of distilled water. A stirring magnet was submerged and the solution was stirred until all the substance was dissolved. The volume was then brought up to 1 liter by addition of distilled water. The same procedure was followed for the monobasic (3.3.3b) buffer, with the difference that 27.6 g/L of NaH_2PO_4 was used.

3.7.2 Extraction solution

A 0.2 M sodium phosphate buffer with pH 6.5 ± 0.2 (extraction solution) (1 liter) was prepared by mixing 315 ml of dibasic sodium phosphate and 685 ml of monobasic sodium phosphate. 10 g of Triton X-100 (3.3.2) was added to a 1 liter beaker (3.2.8). Approximately 500 ml of the sodium phosphate buffer was added to the beaker and stirred until the solution was dissolved (about 20 min). After, 58.4 g of NaCI (3.3.4) was added directly to a 500 ml flask (3.2.5) with addition of ca. 300 ml of sodium phosphate buffer and stirred until dissolved. When the Triton X-100 and NaCl were dissolved, they were mixed into a 1 liter volumetric flask (3.2.5) (total 800 ml of mixture). This mixture was further diluted with sodium phosphate buffer up to 950 ml. Further, the final mixture was stirred until dissolved (around 40 min). The pH was adjusted to the 6.5 ± 0.2, and then sodium phosphate buffer was used to dilute the mixture up to 1 liter.

3.7.3 Substrate solution

A 0.2 M sodium phosphate buffer with pH 5.5 ± 0.2 (0.1 liter) was prepared by mixing 6 ml of the Na_2HPO_4 (3.3.3a) and 94 ml of NaH_2PO_4 (3.3.3b) in a flask (3.2.5) and then diluted with 100 ml of distilled water to make 0.1 M sodium phosphate buffer (substrate solution), 200 ml in total. The mixture was then heated to 30°C in a water bath (3.2.15) and kept in the water bath during analysis. 1.54 g of catechol (3.3.1) was added to the mixture and dissolved right before analysing.

3.7.4 Extraction of PPO

Homogenized berries (3.4.1 or 3.5) were thawed in room temperature, and 10 g were weighed up (3.2.14) and added to a high speed centrifuge tube (3.2.6). Immediately after, 15 ml of reagent A was added. The tubes containing strawberry homogenate and reagent A were kept on ice during this process. Furthermore, the samples were homogenised in a polytron Ultra (3.2.13) for 1 min on 15 rpm while immersed in ice water. Next, the samples were centrifuged (3.2.11) on 14000 x g on 4˚C for 15 min (the samples must rest for 5 min before centrifugation). As a final step, the supernatants were transferred to 50 ml plastic tubes (3.2.7) with a screw cork and kept on ice. The samples were then ready for analysing in the spectrophotometer (3.2.1). The sample extraction was performed in triplicates.

3.7.5 Measurement of PPO activity

200 µl of the extract (3.9.5) were added into a cuvette (3.2.2) and placed in the spectrophotometer (3.2.1). Substrate solution (3.9.3) was added right before the reading of a sample. It is important to start the reading of the sample at once, because the reaction of the catechol will start immediately. Kinect method was used; measurement was taken at 420 nm over a time period of 60 seconds with the measurement interval of 2 seconds. The analysing was performed in duplicates. The slope was determined in the linear range of the reaction curve, which is between 1.1 second to 15.1 seconds. The result was given as absorbance per g sample per minute.

3.8 Colour measurement

Measuring colour was done with HunterLab (3.2.17) (see fig. 10) HunterLab is based on CIE (Commission International e l'Eclairage)L*-, a*-, and b*- values. The L* determines the value for brightness, where white light equals the value 100, a* determines the value for red in the positive direction and green in the negative direction, and b* gives the value for yellow and the positive direction while blue on the negative direction. Before reading of the samples, the instrument was calibrated with a black and white plate.

Fig. 10: Colour measurment equtment (HunterLab)

3.8.1 Procedure

For reading of the samples, a cylindrical measure cell (3.2.22) was used. Cylinder's sides were covered with black rubber to keep light away from the samples. Homogenised strawberries (2.8.1) were filled into the cylinder and a lid was placed on the top. The cylindrical measure cell with the sample was then placed on the instrument (3.2.17), and three readings were made.

3.8.2 Calculations

The result was calculated by averaging of three readings. After reading, the samples were prepared for TMA analysis.

3.9 Total monomer anthocyanins analysis

Principle: Monomeric anthocyanin pigments reversibly change colour with pH; the coloured oxonium form exists at pH 1.0 and the colourless hemiketal form predominates at pH 4.5. The difference in absorbance at 520 nm is proportional to the concentration of monomeric anthocyanins. Results are expressed on a cyanidin-3 glucoside basis. Degraded anthocyanins in the polymeric form are resistant to colour change with pH, hence they are not included in the measurement as they absorb at pH 4.5 as well as pH 1.0 (Lee et al. 2005)

3.9.1 Preparation of reagents

Two buffers were made: (A) potassium chloride (3.3.5) with pH 1.0 and (B) sodium acetate (3.3.6) with pH 4.5.

For potassium chloride buffer solution 1.86 g of KCl was weighted up in a beaker (3.2.8), distilled water was added to 980 ml, and pH was adjusted to 1 with concentrated HCl (3.3.8) (about 6.3 ml was used). The buffer was transferred to a 1 liter volumetric flask (3.2.4) and filled up to 1 liter.

To make 1 liter of Sodium Acetate, 54.43 g CH_3CO_2 Na•$3H_2O$ (3.3.6) was weighted in a breaker (3.2.8) and distilled water was added to ca. 960 ml. The pH was measured and adjusted to 4.5 with concentrated HCl (3.3.8) (about 20 ml was used). The buffer was then transferred to a volumetric flask (3.2.4) and filled up to 1 liter.

Fig. 11: weighing of homogenized berries in high speed centrifuge tubes.

3.9.2 Sample preparation

The amount of around 12 to 13 g of homogenised strawberries (3.4.1 or 3.5), used for the

colour measurement, were added in high speed tubes (3.2.6) (see fig. 11), and centrifuged (3.2.11) at 18000 rpm for 10 min. Directly after centrifuging, the supernatant was removed by a plastic pipette and transferred to a 50 ml tube (3.2.7) and put on ice.

Furthermore, 2 times 200 µl (200 µl per tube) of supernatant were taken out and added to two new tubes (3.2.7). Next, buffer A and B were added to each tube and vortexed. The measurement of absorbent was preformed within 20-50 min of the preparation.

3.9.3 Analysing in the spectrophotometer

Fig. 12: machine used for spectrophotometric analysing

Absorbance must be in the linear range of the spectrophotometer (see fig. 12), between 0.2 – 1.4 nm. The samples were first checked if they were within the range of the spectrophotometer. If needed, the samples were further diluted. The absorbance was measured at 520 and 700 nm.

Note: The reason for measuring at 700 nm is to correct for haze

3.9.4 Calculations

Calculation of anthocyanins concentration was performed by using formula 1. The pigment concentration is expressed as cyanidin- 3 glucoside equivalents, (mg/liter)

$$\frac{A \times MW \times DF \times 10^3}{\varepsilon \times 1}$$

Formula 1

Where A = *($A_{520\ nm} - A_{700\ nm}$) pH 1.0 - ($A_{520\ nm} - A_{700\ nm}$)pH 4.5*; MW (molecular weight) = *449.2 g/mol for cyanidin- 3 glucoside (cyd-3glu)*; DF = *Dilution factor*; ε = *26.900 molar extinction coefficient in* mol^{-1}*, for cyd-3glu*; 10^3 = *conversion from gram to mg.*

4 Results

Table 1 Dry matter (%), pH, Brix, L*, °Hue, Chroma, AF, TMA (mg/100 g ram) and PPO (abs/min/gram) in eight varieties of strawberries, among them two varieties at three degrees of ripeness.

	Dry matter %	pH	Brix	L*	°Hue	Chroma	AF	TMA (mg/100 g sample)	PPO (abs/min/g)
Florence	11.00 ± 0.26 b	3.27 ± 0.00 de	9.90 ± 0.00 d	24,3 ± 0.58 d	33.0 ± 0.3 e	39.9 ± 0.5 abc	1,02 ± 0.01 a	18.1 ± 1.8 de	7.6 ± 0.4 bc
Blink	9.37 ± 0.04 d	3.27 ± 0.00 de	8.03 ± 0.06 f	21,9 ± 0.26 f	35.3 ± 0.5 a	40.4 ± 0.5 ab	0,93 ± 0.02 bc	18.2 ± 1.2 de	5.4 ± 0.3 cde
Sonata	10.54 ± 0.10 bc	3.31 ± 0.01 cd	8.97 ± 0.12 e	27,5 ± 0.53 b	31.0 ± 0.3 g	33.7 ± 0.5 g	0,93 ± 0.01 c	12. ± 1.8 ef	6.7 ± 0.6 cde
GNKv 2078	9.33 ±0.09 d	3.37 ± 0.00 bc	6.73 ± 0.06 g	19,8 ± 0.32 g	33.4 ± 0.3 de	38.4 ± 0.37 de	0,96 ± 0.01 b	40.2 ± 2.1 a	8.4 ± 0.6 b
GNKi 2031	10.35 ± 0.14 c	3.38 ± 0.00 b	9.07 ± 0.06 e	26,3 ± 0.28 c	30.7 ± 0.1 g	36.2 ± 0.3 f	1,02 ± 0.01 a	18.0 ± 3.1 de	7.5 ± 0.8 bc
GNKi 2399	9.29 ± 0.32 d	3.47 ± 0.00 a	7.83 ± 0.06 f	20,6 ± 0.22 g	34.4 ± 0.1 bc	39.1 ± 0.6 bcde	0,94 ± 0.01 bc	34.1 ± 3.0 ab	6.7 ± 0.1 cde
Polka M1	12.09 ± 0.11 a	3.24 ± 0.00 e	10.40 ± 0.00 c	29,5 ± 0.32 a	33.6 ± 0.5 cde	38.3 ± 0.9 de	0,94 ± 0.03 bc	4.2 ± 0.6 fg	7.1 ± 0.6 bcd
Polka M2	12.15 ± 0.10 a	3.32 ±0 .01 bcd	10.67 ± 0.06 ab	25,7 ± 0.35 c	33.7 ± 0.3 cde	38.1 ±0.3 de	0,97 ± 0.01 b	26.3 ± 1.0 bcd	6.7 ± 0.2 cde
Polka M3	12.30 ± 0.08 a	3.34 ± 0.01 bc	10.77 ± 0.06 a	25,6 ± 0.60 c	33.4 ± 0.3 de	40.8 ± 0.4 a	1,03 ± 0.02 a	7.9 ± 2.0 g	6.5 ± 0.4 cde
Senga Sengana M1	12.15 ± 0.10 a	3.28 ± 0.02 de	10.47 ± 0.12 bc	27,1 ± 0.27 b	32.0 ± 0.2 f	35.6 ±0.3 f	0,92 ± 0.01 c	15.2 ± 0.3 e	10.6 ± 1.0 a
Senga Sengana M2	12.27 ± 0.09 a	3.36 ± 0.03 bc	9.97 ± 0.12 d	22,9 ± 0.11 e	34.6 ± 0.4 ab	38.6 ± 0.3 cde	0,94 ± 0.02 bc	22.4 ± 1.7 cde	10.5 ± 0.2 a
Senga Sengana M3	12.21 ± 0.09 a	3.35 ± 0.07 bc	10.80 ± 0.00 a	22,3 ± 0.45 ef	34.1 ± 0.3 bcd	39.3 ± 0.2 cd	0,95 ± 0.01 b	32.2 ± 1.4 abc	10.8 ± 0.4 a

4.1 Quality attributes and chemical composition of frozen strawberry fruits from different cultivars and different degree of ripening

4.1.1 Dry matter

The dry matter content varied from 9.29 to 12.30 in the different genotypes (Table 1). Between the ripening stages the samples were equal. Polka and Senga Sengana had the highest amount of dry matter while GNKv 2399 had lowest amount

4.1.2 PH

The pH level varied in the different strawberry cultivars. Polka ripening stage 1 had the lowest pH (3.24) and GNKv 2399 had the highest pH (3.47) (Table 1). PH levels between maturity stages increased with higher maturity. Polka ripening stage 1 was significantly different from ripening stage 2 and 3 but ripening stage 2 and 3 were equal. The same trend was observed in Senga Sengana.

4.1.3 Brix (soluble solids)

Brix content in the different cultivars varied from 6.37 to 10.80, with GNKv 2078 as the lowest and Senga Sengana with ripening stage 3 as the highest with a difference of 41 %. The two cultivars Polka and Senga Sengana showed that content of Brix significantly increasing during maturation, from the least to the most mature berries

4.1.4 Colour

The parameter of L*, Hue, Chroma and AF are colour parameters from HunterLab analyses. The L* parameter shows the whiteness in the samples, where low values represent darker and high values represent whiter (lighter). The cultivar that was observed to be the darkest was GNKv 2078 with a value of 19.8, while Polka with ripening stage 1 was the lightest with a value of 29.5. Between the maturity stages the berries became darker with higher state of maturity. Polka ripening stage 1 was significantly different from ripening stage 2 and 3 but ripening stage 2 and 3 were equal. The same trend was observed in Senga Sengana.

The parameter of °Hue refers to the colour where 0° to 60° equals red-yellow (the closer to 0° means more bluish-red). The °Hue value varied between the different cultivars, GNKv 2031 had the lowest value with 30.7 and Blink had the highest with 35.3.

Within the various degrees of maturity stages, there was little change in °Hue. The various ripening stages of Polka were equal, but for Senga Sengana the ripening stage 1 was significantly different from ripening stage 2 and 3 but ripening stage 2 and 3 were equal.

The measurements of Chroma showed that Polka M1 had the highest value of with 40.8 while Sonata had the lowest with 33.7. The Chroma in the genotypes with different maturity stages increased from least to most mature. Chroma in Polka increased from 38.3 to 40.8 from M1 to M3 and for Senga Sengana it increased from 35.6 to 39.3.

The AF varied from 0.92 to 1.03 with Senga Sengana as the lowest and Polka M3 as the highest (Table 1). However, Senga Sengana M1 was equal to Senga Sengana M2, Polka M1, GNKv 2399, Sonata and Blink. The AF was increasing with more mature berries. Polka M1 and M2 were significant different from Polka M3. The same was observed for Senga Sengana.

Total monomeric anthocyanins (TMA) content was lowest in Sonata with 12.68 mg/100g and GNKv 2078 had the highest amount of TMA with 40.25 mg/100g (Table 1), which gave a difference of 68 %. Sonata was equal to Florence, Blink, and Senga Sengana maturity stage M1 and M2 and GNKv 2078 were equal to GNKv 2399 and Senga Sengana M3. The amounts of TMA increased with more mature berries. The TMA was increasing with 32 % from stage 1 to stage 2 and 30 % from stage 2 to stage 3 in berries from Senga Sengana.

Polyphenol oxidase (PPO) activity were shown to be highest in the three maturity stages of Senga Sengana, all the three maturity stages were equal (Table 1). Enzyme activity was lowest in Blink, but Blink was also equal to Florence, Sonata, GNKv 2031, 2399, and the 3 maturity stages of Polka.

4.2 Effects of heat treatment, storage time and, cultivar and maturity on PPO activity, TMA content and colour parameters of berry homogenate

Sonata, Senga Sengana M2 and all three maturity stages of Polka were selected for further processing, due to their quality parameters (Table 1). Processing involved crushing berries to homogenous mass, heat treatment and storage at 22 °C for up to 5 weeks. Processed berries were analysed at three time points (no storage, 2 weeks and 5 weeks of storage) to determine PPO activity, TMA content and colour parameters (°Hue, Chroma, L*, AF).

All results are found in Tables 2, 3, 4 and 5. General Linear Model (GLM) was used to perform analysis of variance; results are shown in the upper part of Table 2. Significant effects were found for materials (berries of different cultivars and maturity), heat treatment, storage time and their interactions on all measured parameters, except for TMA that was not significant affected by heat treatment. To give an overview of responses, average values of the main factors (materials, heat treatment and storage time) are shown in the lower part of Table 2. Tables 3, 4 and 5 provide detailed results. Table 3 contains values of TMA and PPO affected by different materials, heat treatment and storage time. Table 4 shows Hue and Chroma values while L* and AF are shown in Table 5.

Table 4. The effects of materials, heat treatment, storage time and their interactions on PPO (abs/min/gram), TMA (mg/100 gram sample) and colour parameters (Chroma, Hue, L, AF). Different small letters in the column for each main effect, denote statistical difference at the level of p<0.05 as determined by Tukey`s comparison test.*

		PPO	TMA	°Hue	Chroma	L*	AF
Materials [a]		P < 0.002	P < 0.001	P < 0.001	P < 0.001	< 0.001	P < 0.001
Heat treatment [a]		P < 0.001	P = 0.771	P < 0.001	P < 0.001	< 0.001	P < 0.001
Storage time [a]		P < 0.001	P = 0.001	P < 0.001	P < 0.001	< 0.001	P < 0.001
Materials*Heat treatment [a]		P < 0.001	P = 0.325	P < 0.001	P < 0.001	< 0.001	P < 0.001
Materials*Storage time [a]		P < 0.001	P < 0.001	0.001	P < 0.001	0.022	0.001
Heat treatment*Storage time [a]		P < 0.001	P = 0.973	P < 0.001	P < 0.001	< 0.001	P < 0.001
Material [b]	Polka M1	6.6 b	3.5 d	34.0 a	26.1 c	30.2 a	0.7 c
	Polka M2	6.8 ab	5.9 c	30.5 d	27.3 b	25.4 c	0.8 b
	Polka M3	7.0 ab	10.1 a	30.5 d	29.4 a	23.3 d	0.8 a
	Sonata	7.2 a	3.6 d	33.5 b	23.7 d	28.8 b	0.6 d
	S. Sengana	6.1 c	7.5 b	31.8 c	28.7 a	25.5 c	0.8 b
Heat treatment [b]	Yes	3.8 b	6.2 a	32.6 a	26.6 b	28.3 a	0.7 b
	No	9.7 a	6.1 a	31.5 b	27.4 a	25.0 b	0.8 a
Storage time [b]	0	7.5 a	12.2 a	30.8 b	36.0 a	27.5 a	1.0 a
	2	6.1 c	4.2 b	29.3 c	24.6 b	25.6 c	0.7 b
	5	6.7 b	1.6 c	36.1 a	20.4 c	26.8 b	0.5 c

Table 5. TMA (mg/100 gram sample) and activity of PPO (Abs/min/gram) in berry homogenate during storage. Different letter denote statistical difference between material at the level of p<0.05 as determined by Tukey`s comparison test.

		TMA			PPO		
	Storage Time (weeks)	0	2	5	0	2	5
Polka m1	Heat Treated (85 °C – 5)	5,94 ± 0,33 efghi	2,59 ± 0,10 ij	0,87 ± 0,09 j	7,08 ± 0,33 ijk	3,54 ± 0,38 lmn	2,11 ± 0,14 mno
Polka m2		10,91 ± 0,57 cd	4,92 ± 0,30 efghij	1,87 ± 0,16 ij	6,10 ± 0,19 jk	2,44 ± 0,03 lmno	2,15 ± 0,01 mno
Polka m3		18.86 ± 0.80 a	8,05 ± 0,16 de	2.93 ± 0,61 fghij	5,61 ± 0,53 k	2,99 ± 0,14 lmno	2,45 ± 0,08 lmno
Sonata		7,54 ± 0,09 defgh	2,55 ± 0,00 ij	0,73 ± 0,11 j	7,66 ± 0,03 hij	3,87 ± 0,33 l	3,58 ± 0,06 lmn
S. Sengana		17,98 ± 0,79 ab	5,06 ± 0,38 efghij	1,48 ± 0,04 ij	3,72 ± 0,23 lm	1,90 ± 0,00 no	1,55 ± 0,20 o
Polka m1	No Heat Treatment	7,80 ± 0,14 def	2,89 ± 0,17 ghij	0,78 ± 0,37 j	8,90 ± 0,09 efgh	7,89 ± 0,12 ghi	10,31 ± 0,35 bcde
Polka m2		11,36 ± 1,72 cd	4,69 ± 0,07 efghij	1,91 ± 0,09 ij	9,35 ± 0,51 defg	9,42 ± 0,51 cdefg	11,42 ± 0,29 ab
Polka m3		19,81 ± 1,09 a	7,66 ± 0,19 defg	2.72 ± 0,05 hij	8,62 ± 0,09 fghi	9,50 ± 0,30 cdefg	12,63 ± 0,09 a
Sonata		8,30 ± 0,78 cde	1,96 ± 0,23 ij	0,64 ± 0,08 j	10,69 ± 0,15 bcd	8,03 ± 0,02 fghi	9,58 ± 0,25 cdef
S. Sengana		13,10 ± 0,03 bc	5,45 ± 0,28 efghij	1,85 ± 0,09 ij	7,18 ± 0,38 ijk	11,05 ± 0,12 abc	11,49 ± 0,72 ab

Table 6. Values of colour parameters (°Hue and Chroma) in berry homogenate during storage. Different letter denote statistical difference between material at the level of p<0.05 as determined by Tukey`s comparison test.

		°hue			Chroma		
	Storage time	0	2	5	0	2	5
Polka m1	Heat Treated (85 °C – 5)	29,99 ± 0,55 klm	31,62 ± 0,21 efghi	41,88 ± 0,03 a	33,69 ± 0,82 ef	23,02 ± 0,70 jkl	19,98 ± 0,64 no
Polka m2		28,85 ± 0,04 mno	28,09 ± 0,10 nop	34,35± 0,07 d	33,39 ± 0,04 ef	25,79 ± 0,12 hi	21,12 ± 0,16 lmno
Polka m3		30,36 ± 0,26 jk	27,83 ± 0,24 op	34,77 ± 0,48 d	36,85 ± 0,15 c	28,23 ± 0,52 g	24,11 ± 1,46 ijk
Sonata		28,80 ± 0,15 mno	32,19 ± 0,36 efg	42,21 ± 0,74 a	31,92 ± 0,66 f	20,59 ± 0,25 mno	17,74 ± 0,91 po
S. Sengana		31,18 ± 0,51 fghijk	30,16 ± 0,11 kj	36,27 ± 0,41 c	35,88 ± 0,08 cd	26,14 ± 0,27 hi	21,40 ± 0,14 lmno
Polka m1	No Heat Treatment	31,56 ± 0,03 efghij	30,73 ± 0,38 hijk	38,24 ± 0,09 b	37,14 ± 0,43 bc	23,12 ± 0,51 jkl	19,46 ± 0,08 op
Polka m2		32,36 ± 0,01 ef	27,11 ± 0,08 p	31,89 ± 0,2 efgh	38,97 ± 0,01 ab	24,43 ± 0,32 ij	19,90 ± 0,05 no
Polka m3		31,75 ± 0,15 efgh	27,52 ± 0,20 p	31,04 ± 0,56 ghijk	39,22 ± 0,31 a	26,80 ± 0,15 gh	21,76 ± 0,19 lmn
Sonata		30,51 ± 0,26 ijk	29,08 ± 0,06 lmn	37,66 ± 0,37 b	34,00 ± 1,05 de	20,11 ± 0,18 no	17,01 ± 0,20 q
S. Sengana		32,41 ± 0,04 e	28,15 ± 0,02 nop	32,30 ± 0,02 ef	39,10 ± 0,12 ab	27,19 ± 0,06 gh	22,28 ± 0,14 klm

Table 7. Values of colour parameters (L and AF) in berry homogenate durin*
g storage. Different letter denote statistical difference between material at the level of p<0.05 as determined by Tukey`s comparison test.

Cultivar		L*			AF		
	Storage time	0	2	5	0	2	5
Polka m1	Heat treated (85 °C – 5)	31,78 ± 0,57 ab	31,83 ± 0,48 ab	34,10 ± 0,34 a	0,97 ± 0,00 de	0,62 ± 0.01 jk	0,35 ± 0.01 r
Polka m2		26,38 ± 0,16 efg	26,27 ± 0,62 efg	29,50 ± 1,64 bcd	1,01 ± 0.00 bc	0,81 ± 0.01 h	0,51 ± 0.00 o
Polka m3		24,38 ± 0,26 ghi	25,25 ± 0,64 fgh	24,87 ± 0,51 fgh	1,05 ± 0.01 ab	0,90 ± 0.01 f	0,56 ± 0.00 mn
Sonata		30,11±0,31 bc	30,19 ± 0,49 bc	30,99 ± 1,31 b	0,97 ± 0.01 de	0,54 ± 0.00 no	0,31 ± 0.01 s
S. Sengana		25,48 ± 0,06 fgh	26,03 ± 0,02 efgh	27,84 ± 0,96 cde	0,99 ± 0.02 cde	0,75 ± 0.01 i	0,47 ± 0.00 p
polka m1	No heat treatment	30,35 ± 0,44 b	26,12 ± 1,01 efgh	27,18 ± 0,18 de	1,00 ± 0.01 cd	0,65 ± 0.00 j	0,40 ± 0.00 q
polka m2		26,65 ± 0,09 efg	21,47 ± 0,29 jk	22,11 ± 0,40 ijk	1,02 ± 0.00 abc	0,80 ±0.01 h	0,53 ± 0.01 no
polka m3		24,49 ± 0,16 ghi	19,85 ± 0,26 k	20,71 ± 0,05 k	1,05 ± 0.01 a	0,86 ± 0.00 fg	0,60 ± 0.01 kl
Sonata		29,44 ± 0,36 bcd	25,06 ± 0,46 fgh	26,53 ± 0,90 efg	0,96 ± 0.02 e	0,61 ± 0.00 kl	0,36 ± 0.01 r
S. Sengana		25,90 ± 0,17 fgh	23,75 ± 0,14 hij	24,27 ± 0,09 ghi	1,02 ± 0.00 abc	0,85 ± 0.00 g	0,58 ± 0.00 lm

4.2.1 PPO

Materials as a factor had significant effect on PPO activities (Table 2) All materials had reduced PPO activity after heat treatment (Table 3). A reduction of around 33 % by average was observed. Of all genotypes, Sonata had the highest activity 5 weeks after the heat treatment was applied whereas Senga Sengana had the lowest. Of all non-heated samples, noteworthy was that Sonata had the lowest activity at 5 weeks. There were no significant differences between the ripening stages of Polka. Storage time was a significant factor, which affected PPO differentially, depending if the heat treatment was applied or not. PPO activity decreased throughout the storage time when heat treatment was applied for all materials. With heat treatment, the most pronounced reduction occurred during the first 2 weeks of storage. In the following 3 weeks PPOs dropped, however, not significantly. The opposite was true for samples that were not exposed to heat. With the exception of Senga Sengana, the general trend was that at 2 weeks, levels dropped a bit or not at all. At week 5, PPO in all non-heated samples increased for approximately 19 %, except for Sonata which remained at the same level.

4.2.2 TMA

Amount of TMA was dependent on the source of material. Of all samples, Polka M3 had the highest while Sonata the lowest amount of TMA at all-time points of storage and irrespective of heat treatment (Table 3). Storage was the factor that had the major effect on the amount of TMA. On average, the amount of TMA fell 62 % from 0 to 2 weeks and an additional 25 % in the following 3 weeks. Importantly, the reduction in TMA was independent of the heat treatment. Within the Polka genotype, maturity level influenced TMA strongly; almost 3 times more was accumulated from M1 to M3.

4.2.3 °Hue

Heat treated samples have lower values of °Hue compared to non-heated samples (Tables 2 and 4). Storage gave the highest changes when the samples were heat treated, with an increase of around 1.3-fold on average after 5 weeks of storage. In non-heated samples at 5 weeks, an increase occurred only for Polka M1 and Sonata. Significant differences in the 3 maturity levels of Polka could be seen at 2 and 5 weeks between M1 on one side and M2 and M3 on the other,

both in heat and non-heat treated samples. The most extreme change was seen for the least mature Polka berries; in heated PolkaM1, °Hue increased from around 30 to almost 42 degrees.

4.2.4 Chroma

Polka M3 and Senga Sengana showed the highest level of Chroma while Sonata was the worst at all-time points (Table 2 and 4). As expected, among Polka samples, the lowest level of Chroma was seen in M1. After 5 weeks of storage, all samples experienced great reduction in Chroma. Heat treatment also contributed to significant reduction of Chroma. Although Polka M3 and Senga Sengana started with equal levels, the latter showed a more pronounced reduction in Chroma after heat treatment. After 5 weeks of storage, there were no significant differences between heated and non-heated samples of the same material. Polka M3 behaved differently though, where higher level of Chroma was measured in heat treated samples compared to non-heated samples.

4.2.5 AF (Acceptance Factor)

Storage time had significantly reduced AF in all samples irrespective of the heat treatment. After 2 weeks of storage only 6 crossed the threshold level of 0.7 and none after 5 weeks. A closer look at heated and non-heated samples revealed that upon 0 weeks the treatment slightly reduced value of AF, yet not significantly. However, heat treated samples stored for 5 weeks showed a more noticeable drop, implying a negative effect of heat treatment on acceptance of strawberry products. In Polka, AF changed significantly with increased maturity level, with a rise of 12 % from M1 to M3.

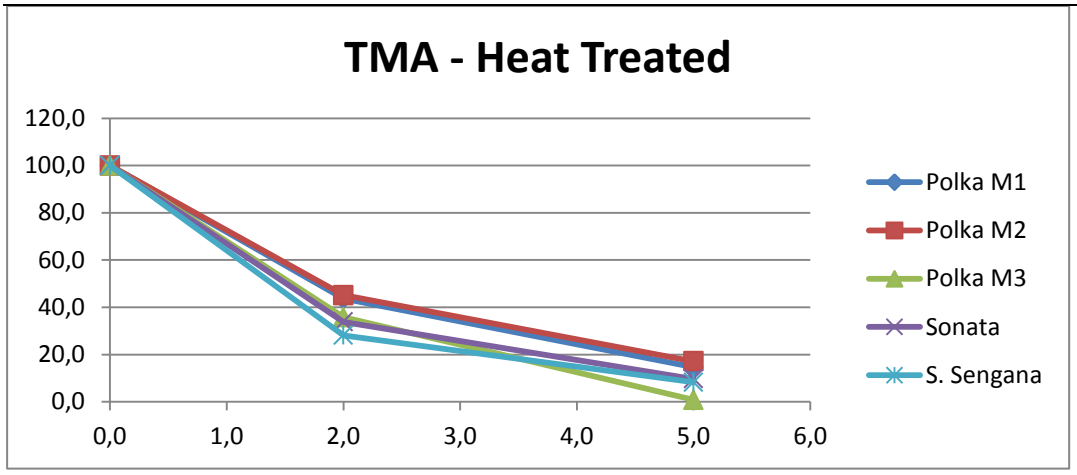

Figure 14 Effect of storage time (0, 2, 5 weeks) in combination with heat treatment (85 °C – 5 min) on content of TMA (mg/100 gram).

Figure 14 shows an illustration of how the TMA content in cultivated strawberries (Polka, Sonata and Senga Sengana, Polka is also represented whit 3 different maturity rates) is affected by storage time in combination whit heat treatment. The changes are presented in percentage. The figure shows that the amount of TMA is decreased rapidly during storage.

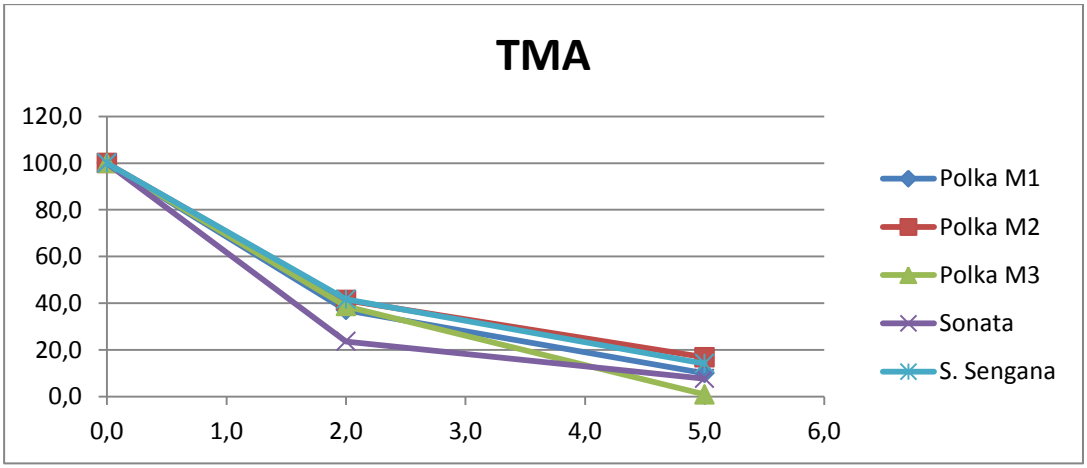

Figure 15 Effect of storage time (0, 2, 5 weeks) on content of TMA (mg/100 gram).

Figure 15 shows an illustration of how the TMA content in strawberries (Polka, Sonata and Senga Sengana, Polka is also represented whit 3 different maturity rates) is affected by storage time. The changes are presented in percentage. The figure shows that the amount of TMA is decreased rapidly during storage.

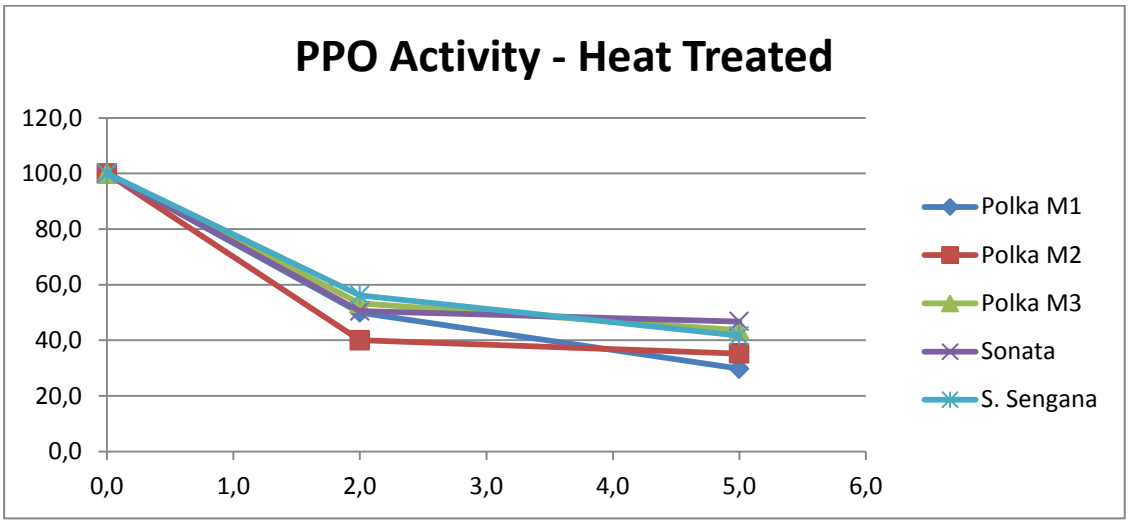

Figure 16 Effect of Storage time (0, 2, 5 weeks) in combination with heat treatment have on the activity of the enzyme PPO.

Figure 16 shows an illustration of how the activity of the enzyme PPO in strawberries (Polka, Sonata and Senga Sengana, Polka is also represented with 3 different maturity rates) is affected by storage time in combination whit heat treatment. The changes are presented in percentage. The Figure shows that the activity is decreased rapidly during the 2 first weeks of storage, and slows down during the next 3 weeks, but the deactivation still occur.

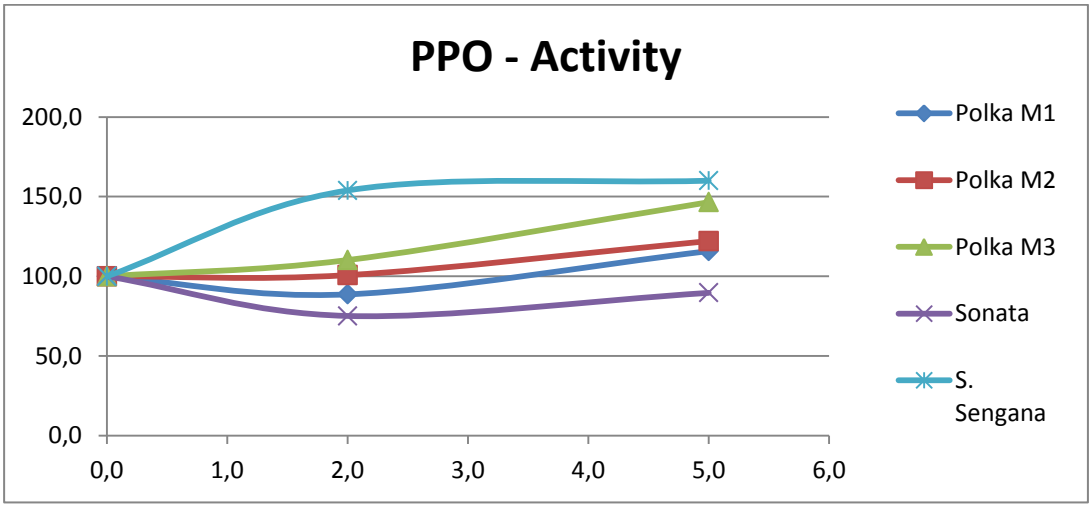

Figure 17 Effect of Storage time (0, 2, 5) have on the activity of the enzyme PPO (abs(min/gram

Figure 17 shows an illustration of how the activity of the enzyme PPO in strawberries (Polka, Sonata and Senga Sengana, Polka is also represented with 3 different maturity rates) is affected by storage time. The changes are presented in percentage. Figure 4 shows that the enzyme

activity is affected differently on the different cultivars. Sonata, Polka M1 and M2 show a deactivation of the enzyme for the first 2 weeks of storage, but then the enzyme gets reactivated. For Polka M3 and Senga Sengana, the enzyme starts to increase form the 2 first week of storage.

5 Discussion

5.1 Effects of genotypes and maturity state

Table 1 presents eight different genotypes and three maturity stages of Polka and Senga Sengana; nearly mature/partly red (M1); mature/red (M2); fully mature/dark red (M3) The cultivars Florence, Blink, Sonata and GNKv 2078, 2031 and 2399 have the maturity stage M2, that is normal, commercial maturity, and when different genotypes are discussed, only maturity stage M2 for Polka and Senga Sengana are considered.

There were significant differences between genotypes for all investigated parameters (dry matter, pH, brix, L*, °Hue, Chroma, AF, TMA, and PPO). Present results are in accordance with a number of previous reports (Skrede et al. 2012, Holzwarth et al. 2012, Yoshida et al. 2002). However, there are not many comprehensive studies and the treatments used in studies published so far differ widely making comparisons difficult at times and any conclusions must be drawn with caution. Skrede at al. (2012) investigated dry matter, pH, brix and TMA in relation to different genetic background. Colour parameters were also reported to be significantly affected by genotypes (Yoshida 2002)(Wrolstad et al. 2008). Holsworth et al. (2012) reported differences in PPO activity for different cultivars, and these generally fell within the range of values found in this thesis. The way affected parameters responded to genotype and maturity different factors is given in the first section of discussion. This is followed by the discussion of the effects of two other factors investigated in this thesis, thermal processing and storage time.

5.2 Dry matter

Dry matter ranged between 7.4 and 10.8 % in the present study. A range between 9.33 and 12.5 % was reported earlier (Skrede et al. 2012) while (Tulipani 2008) found dry matter to range between 5.3 and 12.5 %. This can largely be attributed of the effect different genotypes or by the fact that the literature date used is obtained from diversity of agricultural practices, growing site and climate condition. Dry matter slightly increased during maturation (not significantly), whereas (Tulipani et al. 2011) observed the opposite tendency. This discordance

is obviously due to the fact that the authors chose to study maturity stages at extreme ends (green-red) while the material in this thesis was not as different; nearly mature/partly red (M1); mature/red (M2); fully mature/dark red (M3).

5.3 pH

Differences in pH between genotypes were shown to be small or non-existent at all. It ranged between 3.27 and 3.47 with an average of 3.33. Skrede at al. (2012) found pH in the range 3.41 and 3.74 and Wrolstad at al. (2008) reported a somewhat wider range, between 3.24 and 3.80. It could be concluded that this parameter is quite stable and does not vary very much. The small variation in pH found by different reports is most likely due to the fact that different cultivars were examined.

Montero, et al. (1996) reported a decrease of pH in strawberry fruits during the early stages of fruit development followed by an increase during the latest stage. Present study revealed similar effects of maturation, except for minor inconsistencies seen in Polka and Senga Sengana. pH increased during the maturation for these two cultivars, from M1 to M3. During earlier maturation stages however, pH in Senga Sengana decreased from M1 to M2. On the other side, pH in Polka increased as maturity increased. Polka and Senga Sengana were harvested at different maturity stages, which can probably partly explain the observed effects on pH. When berries are harvested, their maturity stage is determined by evaluating the colour of the fruit and human errors may occur.

5.4 °Brix

Soluble solids (°Brix) content ranged from 6.73 to 10.80. Previous studies found similar values for °Brix (Skrede et al. 2012, Capocasa et al. 2008). Genotype exerted a significant effect on soluble solids. Maturity effect was also quite substantial, as could be expected. °Brix increased in Polka (0.37) and Senga Sengana (0.40) from maturity stage M1 to M3. However, the pattern of increase was different. In Polka, soluble solids increased gradually from the least to the most mature while Senga Sengana showed first a fall of 0.40 from M1 to M2, then a rise of 0.83 from M2 to M3. These apparent differences in the way different cultivars accumulate solids/water

and/or the way they use sugar as they mature, could be due to the limited number of sampling time points. Monero, at al. (1996) showed that soluble solids increased significantly from the point the berry is set until the start phase of maturation. A significant decrease is observed from that time point, but in the very final step of maturation, there is again a slight increase in soluble solids. Present results found the highest levels of soluble solids in the latest maturity stage. As mentioned already, this is possibly due to the limited number of samples, collected only at three time points. It is conceivable that an earlier stage, e.g. between M2 and M3, would show the highest peak for °Brix.

5.5 Total monomeric anthocyanins

The cultivar Sonata contained very low amount of TMA (12.68 mg/100 g) and the cultivar GNKv 2078 had the highest concentration of TMA (40.2 mg/100 g). The other genotypes had in between 18 and 34 mg /100 g of TMA. This is lower than what has been reported earlier (Aaby et al. 2012). Different methods used to determine levels of anthocyanins could easily account for observed differences between studies. In Aaby 2012 at al. anthocyanins were extracted with acetone and quantified after HPLC analysis with pelargonidin-3 glucoside used as an external standard. In the present study, concentration of TMA was determined by the spectrophotometric method on homogenated whole berry samples. Even though the levels of anthocyanins found in this thesis were lower, the ranking of cultivars was according to (Aaby et al. 2012). The only exception was Blink, which was found to have a lower concentration compared to the other cultivars. In contrast, Aaby (2012) found Blink to be one of the cultivars with highest TMA levels. In general, concentration of TMA rose prominently from the least matured to the most mature fruits. For Senga Sengana the concentration doubled from M1 to M3. For Polka, the concentration multiplied over 6 times from M1 to M2, but was reduced almost 4 times from M2 to M3. Previous reports (Aaby et al. 2012, Nunes et al. 2006) showed that TMA increased with maturity rate, and Senga Sengana is clearly in line with these reports. Behaviour of Polka however is not and it remains unexplained. Measurements were repeated; however, the same result was achieved.

5.6 Polyphenol oxidase

PPO activity was highest in Senga Sengana with 10.5 abs/min/gram and the cultivar Blink had the lowest with 5.4 abs/min/gram. The other genotypes showed an activity between 6.7 and 8.4 abs/min/gram. This shows that PPO activity can vary substantially between genotypes. This has been reported earlier in several studies (Holzwarth et al. 2012, López-Serrano and Barceló 2001). In the present study, there was no significant change of the activity of PPO during the three maturity stages. A slight fall in activity from the least matured to the most mature fruits was seen in Polka however. In previous studies (López-Serrano and Barceló 2001, Serradell et al. 2000), it has been shown that PPO activity is at its maximum at "small green" fruits and decreases until the fruits are fully matured. Result presented here did not show this trend. It has to be taken into account that the berries where analysed when the red colour has already started to form.

5.7 Colour parameters

The L*, °Hue, and Chroma values were significantly different between cultivars. The L* value ranged between 19.8 to 25.7. °Hue varied from 30.7 to 34.6 and Chroma from 33.7 to 40.4. There are several different reports that underline these findings (Garcia and Barrett 2002, Yoshida et al. 2002). Parameters were measured on whole berries in these studies, while the berries were homogenised in this thesis. It can be generally concluded that L*, °Hue and Chroma are strongly affected by different genotypes. AF was also affected by cultivars, but there were small differences between cultivars, and all of them were acceptable according to measured AF.

The effects of maturity rate were noteworthy. Lightness (L*) significantly decreased from the least matured to the most mature while Chroma values increased. This has previously been observed by (Nunes et al. 2006). Such pattern shows that the berries become darker and show higher colour saturation with maturity. Nunes, Brecht et al. (2006) investigated three genotypes and showed that °Hue angel decreased during maturation. This is not in accordance with results presented here. In Senga Sengana, an increase in °Hue values by 2.6 between M1

and M2 was significant and was followed by a slight decline from M2 to M3, however, this drop was not significant. Polka showed the same trend. AF had increased from least matured to most matured sample. This is expected, due to the rise in TMA that is known to increase the red colour during the maturation process, hens a* values rises.

5.8 Effects of heat treatment and storage on PPO, TMA and colour of berry homogenate.

As mentioned in chapter "2.3 degradation of phenolic compounds" PPO influences the degradation of anthocyanins that will further affect the colour of strawberry by producing brown pigments. A processing experiment was performed to investigate this. Three genotypes of strawberries were selected; Polka, Sonata and Senga Sengana. Three maturity grades of Polka were included in the analysis. Senga Sengana is the most used strawberry for processing in Norway and Polka is considered as a replacement for Senga Sengana. Sonata was chosen as extreme responses can be expected for this cultivar. It is a cultivar supposedly not suited for processing due to the low amount of TMA. Heated and non-heated materials with and without storage were analysed.

5.8.1 Polyphenol oxidase

The enzyme PPO can be inactivated by heat and pressure treatment (Dalmadi et al. 2006). The authors reported that the PPO activity was reduced by 50 % after heating at 55 °C for 10 min and almost completely inactivated when heated for 10 min at 65 °C. Another study (Serradell, Rozenfeld, at al.) reported that the PPO activity was reduced by 29 % and 57 % after 30 and 120 min at 55 °C, respectively. In present study thermal treatment at 85 °C for 5 min reduced the activity by 20 %, 35 %, 35 % for Polka M1, Polka M2, Polka M3 and 28 % and 48 % for Sonata and Senga Sengana, respectively. The reduction was significant, but lower than expected. As reported earlier, Serradell at al. (2000) and Dalmadi, at al. (2006) showed almost complete inactivation of the enzyme after 10 min at 65 °C. It is stated that samples in the present study were heated at 85 °C for 5 min, but it is important to know that it took 15 min before the samples reached 85 °C. It could thus be assumed that the samples were at least for 7-8 min over 65 °C. This can be considered as a harder heat treatment compared to 10 min at 65 °C. Therefore, the expected results were that the enzyme would be almost completely inactivated.

However, in the present study the enzyme showed residual activity as follows 80 %, 65 %, 65 %, 72 % and 52 % for Polka M1-M3, Sonata and Senga Sengana, respectively, when compared to non-heat treated samples. This indicates that the thermal treatment at 85 °C for 5 min is not sufficient to completely inactivate PPO. In addition different cultivars and maturity state of Polka show different levels of enzyme activity following the heat treatment. Conflicting results were previously reported (Dalmadi et al. 2006, López-Serrano and Barceló 2001). Taken together, interactions between genotypes and maturity stages can be expected to have an effect on PPO inactivation by heat.

An unexpected finding was the combined effects of storage and heat treatment. After 2 weeks of storage, PPO activity was reduced by 55 %, 74 %, 69 %, 52 % and 83 % for Polka M1-M3, Sonata and Senga Sengana, respectively, when compared to non-heat treated samples stored for 2 weeks. After the additional 3 weeks of storage, the ratio between heat and non-heat treated samples have risen; 80 %, 81 %, 81 %, 63 % and 87 % for Polka M1-M3, Sonata and Senga Sengana, respectively. Thus, heat treatment combined with storage induced deactivation of PPO throughout the storage time. On the other hand, non-heated samples showed a reduction of activity at two weeks of storage, but after five weeks PPO activity had risen (reactivated PPO). This indicates several things. Firstly, genotypes and maturity stage respond differently to the heat treatment; Sonata had shown significantly lower reduction of the PPO activity compared to the other two genotypes. Secondly, maturity of Polka affected PPO activity during the first 2 weeks only. After 5 weeks, the maturity stages of Polka showed almost identical inactivation of the enzyme. Without the heat treatment, PPO activity had higher activity after 5 weeks of storage, whereas with heat treatment, a major deactivation throughout the storage period occurred. Of note is that PPO exhibited high thermal stability in this study, which was in accordance with previous reports (Dalmadi et al. 2006, Holzwarth et al. 2012, López-Serrano and Barceló 2001). Earlier reports suggested the involvement of PPO in the degradation of TMA (Dalmadi et al. 2006). Based on this, higher levels of TMA were expected in non-heated stored samples.

5.8.2 Total monomeric anthocyanins

Present results showed that inactivation of PPO had no significant effect on levels of TMA. Interestingly, Holzwarth et al. (2012) reported similar findings in the two genotypes, Elsanta and Everest. The study suggested that the enzyme might have been reactivated during storage. Similarly, samples in our study showed residual PPO activity upon 2 and 5 weeks of storage but there were no reactivations. However, the residual PPO activity might explain TMA reduction, especially the fact that only 33 % reduction in PPO activity upon thermal treatment was achieved seems important. This implies partial deactivation of PPO and that heat treatment for 5 min at 85 ° is not sufficient to maintain TMA in stored strawberry purees. Furthermore, these findings suggest that the level of colour degradation might be similar between heated and non-heated samples. Surprisingly however, colour analyses showed that PPO inactivation by heat treatment had a negative effect on colour parameters.

5.8.3 Colour measurements

°Hue levels were higher in heated samples than in non-heated samples after 5 weeks of storage, indicating that heated samples were turning yellow(ish). The parameter of Chroma showed almost the same trend between heated and non-heated samples; only Polka M3 showed a significant difference between heated and non-heated samples. Value of L* were significantly higher in heat treated samples, which means heat treated samples turned brighter than non-heated samples. And finally, AF was lower in heat treated samples, showing that heat treated samples were less attractive for customers. However, AF levels were way below 0.7 for both heat and non-heat treated samples. This shows that heat treatment is not an ideal treatment for preserving colour in strawberry puree.

Interestingly, Polka M1 and Sonata had similar responses to processing. The materials Polka M1 and Sonata were equal before storage and had the lowest level of °Hue, in both heat and non-heat treated samples, and after 5 weeks of storage, they were still equal and had the highest level of °Hue in both heated and non-heated samples. This was evidence that Polka M1 and Sonata changed more rapidly in the colour spectra during storage then other materials. An explanation for this can be that these two samples had the lowest amount of TMA and the highest L*. Such conditions would make brownish pigments more visible.

In summary, profiling eight strawberry genotypes revealed that it is difficult to find one cultivar that will be best suitable for processing. However, two genotypes Sonata and GNKv 2078, were found to exhibit non-desirable and desirable processing characteristics, respectively. Sonata was found to be the least suitable, when colour parameters and amount of TMA were studied and GNKv 2078 was found to be the most suitable. Among the other six cultivars, it was difficult to point out which ones would be the most suitable for processing. During the processing experiment the goal was to find a cultivar that maintains TMA and its natural colours during storage. As expected, Sonata was found to be the least suitable for processing and Polka and Senga Sengana were found to respond similar

6 Conclusion

Amount of TMA, PPO activity and maintaining the natural colour of strawberry homogenate during storage have been the focus of this thesis. Between the genotypes it was found that Sonata had the least favourable initial quality parameters for processing, and during the processing experiment this was shown to be true. There were only small differences between Polka and Senga Sengana. More mature fruits were better suited for processing than less mature fruits. Fruits with higher contents of TMA were more suited for processing than fruit with low content.

Heat treatment of strawberry negatively affected colour parameters but had no effect on concentration of TMA. PPO was to a certain degree inactivated by heat treatment. Significantly higher PPO activity could be measured in non-heated samples after long storage. This however, did not prevent degradation of TMA during storage. Thus, heat treatment is not suitable for inactivation of PPO. Heat treatment should be as gentle as possible for slowing down spoilage caused by microbial growth, but other methods should be used to inactivate PPO, e.g. pressure or enzyme inhibitors. Finally, the storage time had an enormous negative effect on TMA and colour parameters.

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