JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Processing Strawberries to Different Products Alters Contents of Vitamin C, Total Phenolics, Total Anthocyanins, and Antioxidant Capacity

YVONNE KLOPOTEK,† KONRAD OTTO,‡ AND VOLKER BÖHM*,†

Institute of Nutrition, Friedrich Schiller University Jena, Jena, Germany, and Institute of Beverage Technology, University of Applied Sciences Lippe and Höxter, Lemgo, Germany

Strawberries were processed to juice, nectar, wine, and puree. For investigation of the antioxidant capacity as well as the contents of ascorbic acid, total phenolics and total anthocyanins, samples were taken after different stages of production to determine the effects of processing. The content of vitamin C was measured spectrophotometrically. The total phenolic content was analyzed by using the Folin−Ciocalteu method, and the amount of total anthocyanins was determined by using the pH-differential method. Two different methods—the trolox equivalent antioxidant capacity assay and the ferric reducing antioxidant power test—were used to determine the hydrophilic antioxidant capacity. This study showed the decrease of all investigated parameters within processing strawberries to different products. The content of ascorbic acid decreased with production time and processing steps, especially during heat treatment. The investigations on total phenolics in strawberry products proved fining to be a mild method to clarify berry juices and wines without removing high amounts of total phenolics. Fermentation did not lead to heavy losses of total phenolics, probably due to polymerization and condensation of monomer phenolics such as anthocyanins. Total anthocyanins and the hydrophilic antioxidant capacity decreased while using high temperatures. Anthocyanins also decreased considerably during the processing of wines, mainly caused by fermentation and pasteurization.

KEYWORDS: TEAC assay; FRAP assay; hydrophilic antioxidants; strawberry juice; strawberry wine; strawberry puree

INTRODUCTION

A high dietary intake of fruits and vegetables is supposed to reduce the risk of coronary heart disease and cancer (*1*−*3*). The positive effects of fruits and vegetables may be associated with their high amounts of several secondary plant products (*4*−*7*). Strawberries are rich in water-soluble vitamin C. Apart from that, they contain certain compounds of the extensive class of polyphenols as phenolic acids such as *p*-cumaryl−glucoside and *p*-hydroxy-benzoic acid−glucoside and flavonoids (*8*). In addition, anthocyanins are very important substances in strawberries (*9*). Anthocyanins are responsible for the red and blue colors of many fruits and berries such as black currants, raspberries, strawberries, and red grapes (*10*, *11*). The most important anthocyanin in strawberries is the water-soluble pelargonidine-3-glucoside (*12*). Vitamin C and phenolic compounds are able to scavenge oxygen radicals and to avoid the formation of oxidative stress. All these substances with their high antioxidant capacities contribute to the quality of strawberries and their products (*13*). Thus, it is very important to reduce losses of healthy constituents during processing.

Investigations of strawberries mainly deal with their phenolic compounds and antioxidant capacity. Studies about fruit processing and its influence on antioxidant substances are scarce. Waste products such as pomace or suspended particles were rarely noted.

Strawberries are consumed mainly as fresh fruit. In addition, many other strawberry products such as juice, nectar, puree, and juice concentrate as well as jam are commercially available. Common processing steps are the concentration of fruit juice, storage in tank farms, redilution, production of strawberry jam by heating under vacuum, bottling, closing under vacuum, and cooling (*14*).

In this study, strawberries were processed to juice, nectar, puree, and wines under industrial-like conditions but without adding ascorbic acid. The first wine was produced by fermenting the mash, and the second was produced by fermenting centrifuged juice. Samples of almost every production step including samples of pomace and suspended particles were taken to perform a stepwise control and were analyzed on their contents of vitamin C, total phenolics, total anthocyanins, and antioxidant capacity. The results of these investigations could be useful for the fruit juice industry to avoid processing steps and product treatments that lead to a high reduction of the antioxidant capacity. The additional measurements of waste products might

* Corresponding author phone: +49 3641-949633; fax: +49 3641-949632; e-mail: Volker.Boehm@uni-jena.de.
† Friedrich Schiller University Jena.
‡ University of Applied Sciences Lippe and Höxter.

10.1021/jf047947v CCC: $30.25
Published on Web 06/08/2005

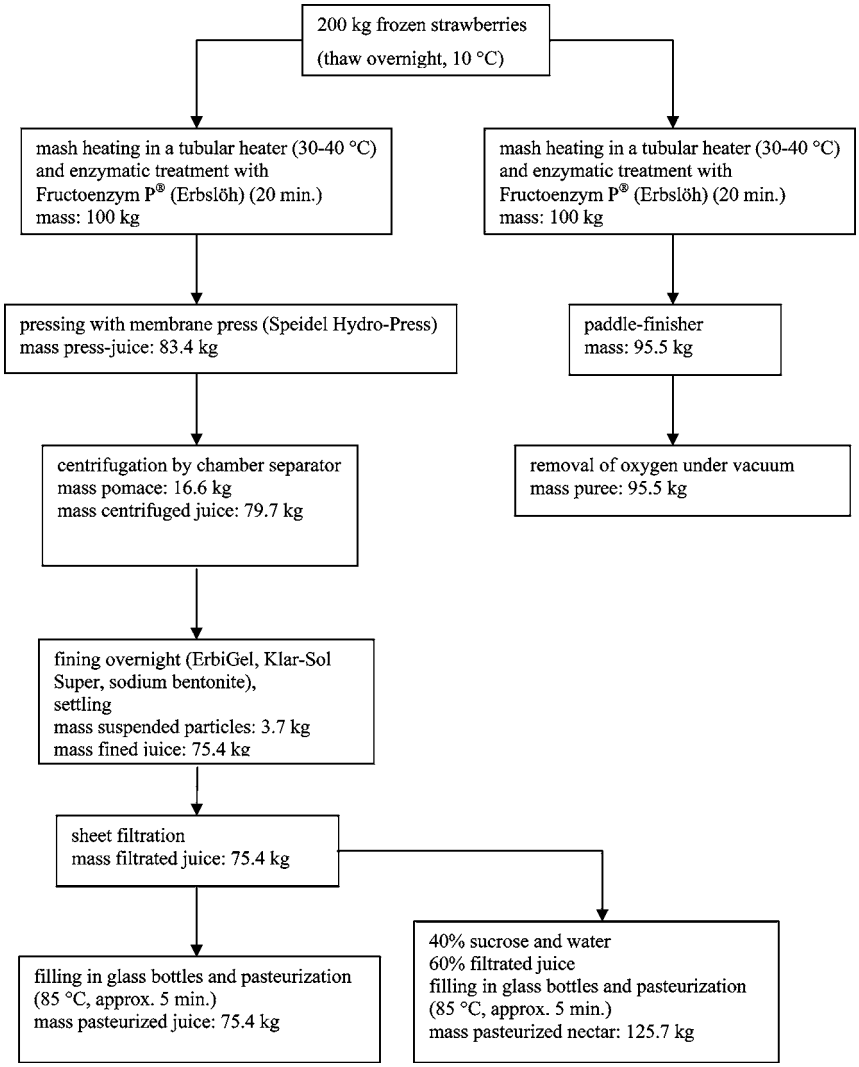

Figure 1. Processing strawberries to juice, nectar, and puree.

be of interest to influence the production process to save more important substances.

MATERIALS AND METHODS

Chemicals. All chemicals used were of analytical grade. Special reagents were ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) (Sigma no. A 1888, Sigma-Aldrich, Taufkirchen, Germany), myoglobin (Sigma no. M 1882), Trolox ((*S*)-(−)-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) (Aldrich no. 39,192-1, Sigma-Aldrich, Taufkirchen, Germany), Folin−Ciocalteu's phenol reagent (Fluka no. 47641, Sigma-Aldrich, Taufkirchen, Germany), TPTZ (2,4,6-tripyridyl-*s*-triazine) (Sigma no. T 1253), and DNP (2,4-dinitrophenylhydrazine) (Fluka no. 42210).

Sample Preparation. The production processes were carried out at the University of Applied Sciences Lippe and Höxter from frozen strawberries (*Fragaria* × *ananassa* var. Polka). Samples were taken after almost each stage of production (**Figure 1**). Fructoenzym P (Erbslöh, Geisenheim, Germany), a pectolytic enzyme preparation (pectinesterase, pectinlyase, and endo-polygalacturonase), was used for the enzymatic treatment of the mash. The wines were produced by mash fermentation and from centrifuged juice. After this, the wines were centrifuged, fined, filled in bottles, and pasteurized. Samples were taken from the final wines (**Figure 2**).

All samples were stored at −18 °C until analysis. Nonliquid samples were homogenized before analysis.

Measurement of Ascorbic Acid. The vitamin C content was analyzed photometrically after oxidation (catalyzed by copper ions) of ascorbic acid to dehydroascorbic acid, which reacts with 2,4-dinitrophenylhydrazine (DNP) to form a red complex. The absorbance was measured at 520 nm (*15*). The 0.3−0.6 g sample was mixed with 5 mL of meta-phosphoric acid and vortexed for 1 min. After centrifugation of the mixture, the liquid layer was transferred into a flask. This extraction was repeated twice for liquid samples such as juice or nectar and 4 times for nonliquid samples, for instance strawberries or mash (*16*). A total of 200 μL of the sample was mixed with 300 μL of trichloroacetic acid and centrifuged (12 000 rpm) for 5 min. Afterward, 100 μL of DNP reagent (copper sulfate, thiourea, and DNP) was added to 300 μL of the liquid layer and later mixed. The solution was heated (60 °C) and shaken for 1 h. Before adding 400 μL of sulfuric acid, the sample was cooled for 5 min on ice. The samples were kept in the dark for 20 min before the absorbance was measured at 520 nm. Ascorbic acid solutions were used for calibration (*15*).

Determination of Total Phenolic Content. The content of the total phenolics was evaluated by using the Folin−Ciocalteu method. A total of 0.2−0.4 g of each sample was weighed in a 10 mL flask and filled up with distilled water. The extract was centrifuged (5000 rpm) for 5 min. The 200 μL sample solution was mixed with 800 μL of Na_2CO_3 solution and 1 mL of Folin reagent. The samples stood for 120 min at room temperature before the absorbance was measured at 750 nm. Gallic acid monohydrate was used as a standard, and the total phenolic content was expressed as gallic acid equivalents (GAE) (*17*).

Determination of Total Anthocyanin Content. The total anthocyanin content was determined by using a modified pH-differential method. A total of 1−2 g of the samples was weighed. Three milliliters of a solution mixture consisting of 2 parts formic acid and 28 parts water was added to nonliquid soluble samples. After 5 min of standing,

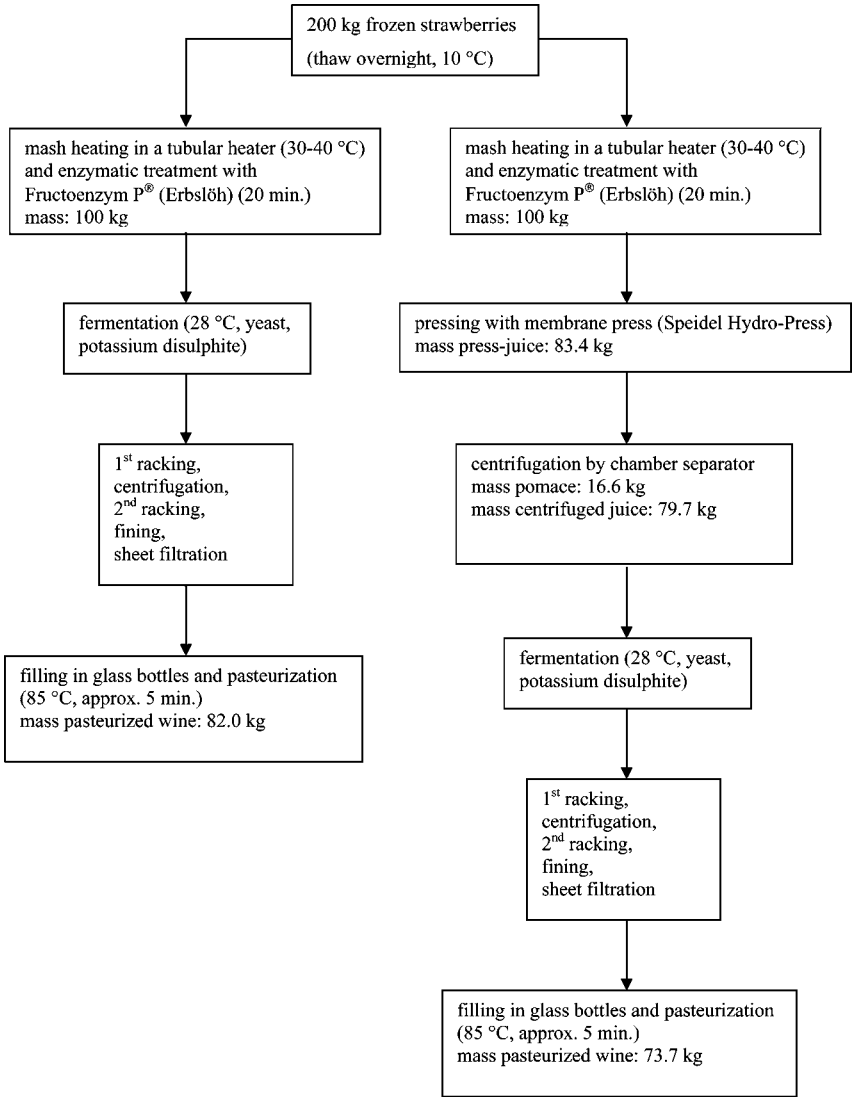

Figure 2. Processing strawberries to wine, made from mash and centrifuged juice.

the next step was the addition of 3 mL of methanol. The solution was shaken for 1 min. The liquid samples were treated with 5 mL of a solution mixture consisting of 70 parts methanol, 2 parts formic acid, as well as 28 parts water. They also were allowed to stand for 5 min. All samples were shaken for 1 min and frozen (−18 °C) for 15 min before they were centrifuged (5000 rpm). The liquid layer was transferred into a 10 mL flask. Nonliquid samples were filled up with methanol, whereas liquid samples were filled up with the solution mixture, followed by a 5 min centrifugation (5000 rpm) (*18*). The absorbance values were measured at 515 and 700 nm in buffers, each at pH 1.0 and 4.5 (*19*). Results were calculated according to the equation mentioned next and converted to milligrams of pelargonidin-3-glucoside (PG-3-G) per 100 g of fresh weight.

$$E = (E_{515} - E_{700})_{\mathrm{pH1,0}} - (E_{515} - E_{700})_{\mathrm{pH4,5}}$$

$$c\ (\mathrm{mg/L}) = (E \times \text{molecular weight} \times \text{dilution factor} \times 1000)/(\epsilon L)$$

The molecular weight of pelargonidin-3-glucoside is 433.0 g/mol, and ϵ is 22 400 mol^{-1}.

Ferric Reducing Antioxidant Power (FRAP) Assay. The FRAP test is based on the reducing potential of antioxidant substances. Together with distilled water, the sample solution was pipetted into a microtiter plate. In the next step, the FRAP solution was added and 8 min after starting the reaction, the absorbance was measured at 595 nm. During that reaction, the iron(III) complex was reduced to the iron(II) form, and ferrous sulfate solutions were used for calibration (*20*).

Trolox Equivalent Antioxidant Capacity (TEAC) Assay. The hydrophilic antioxidant capacity was performed by using the $ABTS^{\bullet+}$ radical cation assay. In the presence of metmyoglobin and hydrogen peroxide, ABTS is oxidized to the durable radical cation $ABTS^{\bullet+}$ that is measured photometrically at 734 nm. Trolox was used as the standard, and the antioxidant capacity was expressed as trolox equivalent antioxidant capacity (*13*, *20*, *21*).

Statistical Analysis. All data were reported as means ± standard deviation of three samples with two replications each ($n = 6$), taken from one process. Statistical analysis was performed using SPSS software (SPSS for WINDOWS 10.0, SPSS Inc. Chicago). Differences between variables were tested for significance by using the ANOVA procedure (Tukey), using a level of significance of $p < 0.05$.

RESULTS

Vitamin C. The water-soluble vitamin C decreased during the production process of juice and nectar, which is shown in **Table 1**. The raw strawberries used in this study showed a vitamin C content of 104 ± 2 mg/100 g of fresh matter. The biggest losses of vitamin C were caused by pressing and pasteurization. The press process led to a loss of about 22% of the vitamin C concentration. The main part (18%) of the total loss was caused by pomace removal (as compared to mash). The heat treatment destroyed the heat instable vitamin C and led to a decrease (as compared to filtrated juice) of 35% in juice and 28% in nectar (**Table 2**). Fining had a minor influence on

Table 1. Alterations of Measured Parameters during Processing of Strawberries to Juice, Nectar, Puree, and Wine[a]

samples	explanation on related processing steps					vitamin C (mg/100 g or %)	total phenolics (mg/100 g or %)	total anthocyanins (mg/100 g or %)	FRAP (μmol/100 g or %)	TEAC (μmol/100 g or %)
strawberries	J1[b]	N1	P 1	WM1	WJ 1	104.1 ± 1.7^{Ac}	257.1 ± 2.4^{A}	42.2 ± 0.3^{A}	2466 ± 97^{A}	1190 ± 30^{A}
mash	J2	N2	P 2	WM2	WJ 2	96.2 ± 1.4^{B}	85.4 ± 1.3^{B}	120.2 ± 6.1^{B}	91.7 ± 4.9^{B}	90.6 ± 7.2^{B}
press-juice	J3	N3			WJ 3	75.5 ± 1.4^{C}	61.0 ± 0.6^{C}	101.9 ± 4.4^{A}	69.7 ± 1.9^{C}	70.9 ± 2.7^{C}
pomace	J4	N4			WJ 4	17.2 ± 0.7^{D}	17.5 ± 0.4^{D}	23.3 ± 0.6^{C}	17.3 ± 1.0^{D}	18.2 ± 0.6^{D}
centrifuged juice	J5	N5			WJ 5	65.8 ± 1.1^{E}	53.4 ± 1.3^{E}	103.1 ± 5.0^{A}	58.9 ± 3.6^{E}	60.3 ± 1.4^{E}
suspended particles	J6	N6				3.8 ± 0.1^{F}	2.5 ± 0.1^{F}	6.4 ± 0.2^{D}	2.7 ± 0.2^{F}	3.0 ± 0.3^{F}
polished juice	J7	N7				63.6 ± 0.6^{G}	49.8 ± 0.4^{G}	90.1 ± 0.4^{E}	55.0 ± 2.9^{E}	60.8 ± 3.1^{E}
filtrated juice	J8	N8				55.3 ± 2.9^{H}	49.0 ± 0.5^{H}	92.3 ± 1.9^{F}	51.6 ± 1.4^{G}	55.9 ± 2.9^{G}
pasteurized juice	J9					36.0 ± 1.9^{I}	35.6 ± 0.5^{I}	67.3 ± 6.0^{G}	41.8 ± 2.1^{H}	34.4 ± 2.3^{H}
pasteurized nectar		N9				39.8 ± 1.1^{J}	42.1 ± 0.3^{J}	56.8 ± 1.8^{H}	48.1 ± 2.8^{I}	47.0 ± 0.8^{I}
puree			P3			89.0 ± 1.4^{K}	73.6 ± 0.9^{K}	117.6 ± 8.6^{B,I}	76.3 ± 4.2^{J}	87.4 ± 2.0^{B}
puree (degassed)			P4			88.4 ± 1.4^{K}	69.5 ± 1.2^{L}	113.3 ± 1.4^{I}	66.3 ± 3.0^{K}	79.6 ± 1.9^{J}
wine (from mash)				WM3		36.2 ± 2.3^{L}	62.6 ± 0.8^{M}	30.8 ± 1.4^{J}	71.2 ± 3.2^{L}	69.1 ± 3.3^{K}
wine (from juice)					WJ6	34.0 ± 2.1^{M}	46.9 ± 0.9^{N}	20.7 ± 0.7^{K}	50.3 ± 3.8^{M}	56.5 ± 3.8^{L}

[a] For strawberries, results are given as absolute values in fresh matter, and for all other samples, results are given as relative values (%) related to strawberries (=100%) [b] J1–J9: necessary processing steps for juice production. N1–N9: necessary processing steps for nectar production. P1–P4: necessary processing steps for puree production. WM1–WM3: necessary processing steps for wine production made of mash. WJ1–WJ6: necessary processing steps for wine production made of centrifuged juice. [c] A–N: values with different superscript letters within one column are significantly different ($p < 0.05$).

Table 2. Effects of Pasteurization on Contents of Vitamin C and Total Phenolics while Producing Juice and Nectar[a]

	filtrated juice	final product	processing loss (%)
vitamin C (g/100 g)			
strawberry juice	1.10 ± 0.06^{Ab}	0.72 ± 0.04^{B}	35.0
strawberry nectar	1.10 ± 0.06^{a}	0.79 ± 0.02^{b}	28.0
total phenolics (g/100 g)			
strawberry juice	2.41 ± 0.03^{A}	1.76 ± 0.03^{B}	27.2
strawberry nectar	2.41 ± 0.03^{a}	2.07 ± 0.02^{b}	14.1

[a] Results are given as absolute values in dry matter. [b] A–B and a–b: values with different superscript letters within one row are significantly different ($p < 0.05$).

the loss of vitamin C. Processing to puree led to a vitamin C decrease by 12% as compared to raw strawberries. The production step of removing oxygen did not lead to significant differences (**Table 1**). The fruit wine produced of mash showed a total loss of 64% vitamin C, comparable to the wine (−66%) produced from centrifuged juice (**Table 1**).

Total Phenolics. The changes of the total phenolic contents during the processing of strawberries to juice and nectar are shown in **Table 1**. The strawberries had 257 ± 2 mg of GAE/100 g of fresh matter. The phenolic compounds decreased with advanced time of processing and stage of production. One step with high influence on the contents of phenolics was the pasteurization of juice (−27%) and nectar (−14%), shown in **Table 2**, accompanied by the mashing and the pressing process with a loss of 15 and 29%. Fining and filtration had only a minor influence on the content of phenolics. **Table 1** shows the changes of total phenolic content in strawberries to puree. The amount decreased from 100% in strawberries to 70 ± 1% in puree. The largest loss of phenolics was caused by mashing. Similar amounts of phenolics were measured in one of the wines. The wine produced from centrifuged juice had only a content of 47% of the total phenolics as compared to strawberries (**Table 1**).

Total Anthocyanins. The total anthocyanin content changed from 100% (42.2 ± 0.3 mg of PG-3-G/100 g of fresh matter) in strawberries to 67 ± 6% in pasteurized juice and 57 ± 2% in heated nectar. The process showed surprising results of increasing contents of anthocyanins at the beginning of the production (mash as compared to strawberries). During the following processing steps, the content of anthocyanins remained nearly unchanged (**Table 1**). The pasteurization led to a decrease (related to filtrated juice) of 27% in juice and 39% in nectar. During the production of puree, the alterations of the anthocyanin content were modest. Passing the mash through a sieve did not result in significant changes. The oxygen-free puree had the same content of anthocyanins as the nonvacuum treated puree (**Table 1**). The final strawberry wines showed high losses (as compared to strawberries) of 69 and 79% anthocyanins (**Table 1**).

Antioxidant Capacity. The measured hydrophilic antioxidant capacity values were 1190 ± 30 μmol of TE/100 g (TEAC assay) and 2466 ± 97 μmol of Fe^{2+}/100 g of fresh strawberries (FRAP assay). Using the FRAP assay as well as the TEAC method, the results of the antioxidant capacity of juice and nectar showed a similar decrease within the processing procedure of the strawberries (**Table 1**). The antioxidant capacity of strawberry puree determined as a FRAP value decreased by 34% during the process. Passing the mash through a sieve was the step causing the largest decrease of antioxidant capacity. As compared with the results obtained with the TEAC assay, there were noticeable differences. The loss of the trolox equivalent antioxidant capacity was only 20%, and the process of making puree from mash did not result in significant changes (**Table 1**). The strawberry wines showed comparable high values of antioxidant capacity. Especially, the wine made of mash contained 71% (FRAP) or 69% (TEAC) of the antioxidant capacity as compared to strawberries (**Table 1**).

DISCUSSION

As expected, the content of vitamin C decreased during the processing of strawberries. The vitamin C values depend on the species and on cultivation conditions (*22*). The strawberries used in this study showed a vitamin C content (**Table 1**) that is higher but still comparable to existing data from Proteggente et al. (*12*), who analyzed contents of vitamin C of 61 mg/100 g of fresh strawberries. The water-soluble vitamin C passed into the liquid phase, but also a certain part was lost with pomace and suspended particles. The production steps with the highest influence were the pasteurization as well as mashing or finishing. On the basis of dry matter, the nectar showed a lower loss of vitamin C than the juice. This difference between the final products might be caused by the pasteurization procedure, which

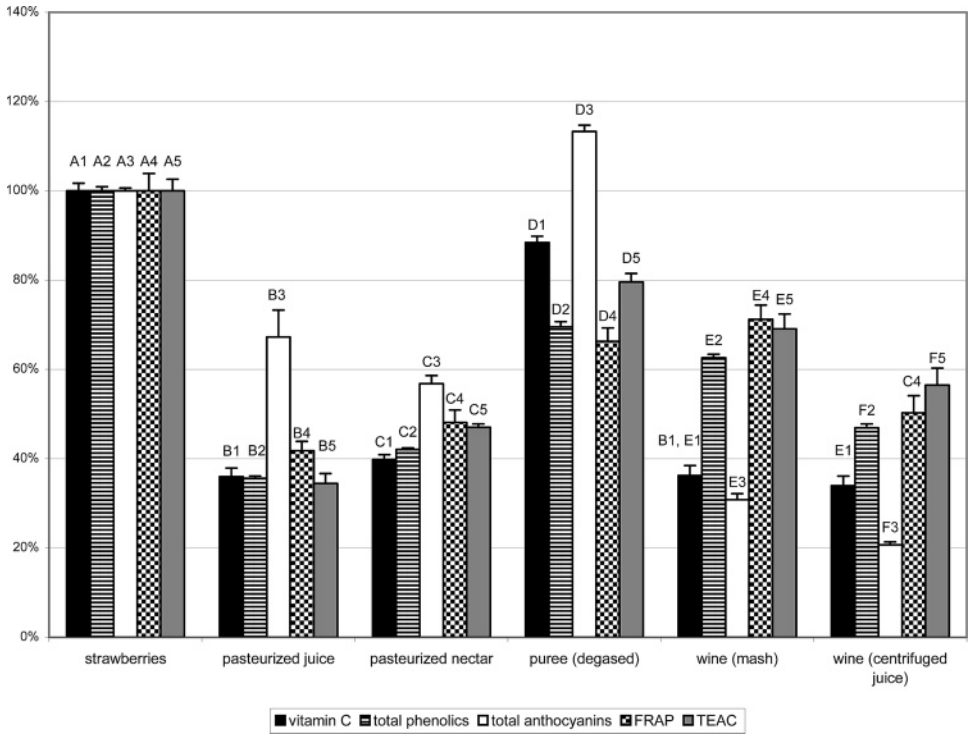

Figure 3. Alterations of measured parameters in strawberries and final products (juice, nectar, puree, and wines). A1–E1: vitamin C values with different superscript letters are significantly different ($p < 0.05$). A2–F2: total phenolic values with different superscript letters are significantly different ($p < 0.05$). A3–F3: total anthocyanin values with different superscript letters are significantly different ($p < 0.05$). A4–E4: FRAP values with different superscript letters are significantly different ($p < 0.05$). A5–F5: TEAC values with different superscript letters are significantly different ($p < 0.05$).

took place at 85 °C for 8 ± 3 min. The juice and the nectar were not pasteurized at the same time. Therefore, the heating procedure might have been slightly different. Vitamin C, which is unstable to heat and oxygen, is oxidized to nonantioxidant effective substances. Additional time-consuming treatments such as fermentation of the mash and centrifugation of the juice to receive wine reduced the vitamin C content because enzymes and oxygen are capable of attacking and inactivating vitamin C.

Phenolic compounds measured in strawberries confirmed results from other studies (*12*, *23*, *24*). Proteggente et al. (*12*) determined the total phenolic content of strawberries with 330 mg of GAE/100 g of fresh matter, and Heinonen et al. (*23*) measured only half of that result (161 mg of GAE/100 g of fm). The own result of total phenolics in strawberries with 257 ± 2 mg of GAE/100 g of fresh matter is in a similar range. However, different species contain varying concentrations of total phenolics (*24*). The processing steps heat treatment, mashing, and fermentation led to extensive losses of phenolic substances. In contrast, the filtration and fining had only minor effects. The fining of berry juices caused modest changes of the phenolic content, which is also mentioned by Rechner (*9*). As compared to the production of juice, nectar, and puree, it was surprising that in wines high amounts of phenolic substances remained, although there were much more production steps including longer processing times.

Anthocyanins are the most important phenolic substances in strawberries and belong to the flavonoids (*10*, *12*, *25*). The content of total anthocyanins in the strawberries (**Table 1**) used within this study was higher than in other studies or similar to existing data (Wang et al. (*26*): 38.9 mg/100 g of fresh matter), which is an indicator for high variations (*27*−*29*). Clifford et al. mentioned values of 15−35 mg/100 g of fresh matter (*27*), Macheix et al. published results of 28−70 mg/100 g of fresh strawberries (*28*), and Meyers et al. measured 21.9−41.4 mg/100 g (*24*). The anthocyanin contents showed only little changes and even an increase from strawberries to mash during the processing of strawberries that might be caused by extraction restrictions in raw strawberries. These results are comparable to the data found by Rechner (*9*). One explanation for minor alterations of the anthocyanin content could be a continuous release of anthocyanins out of cell fragments (*9*, *29*). Also, copigmentation of the colored flavylium cation by phenolic substances such as flavonoids or phenolic acids might be possible (*9*, *30*−*34*). Self-association of the flavylium cation, which forms a hydrophobic core surrounded by hydrophilic glycosyl rests, probably occurs (*30*−*32*). The phenolic components react with the flavylium cation by using hydroxyl groups. Because of these reactions, the colored flavylium cation is stabilized (*30*−*33*). The content of total phenolics, measured by using the Folin−Ciocalteu method, decreased during the processing steps. The number of hydroxyl groups affects the amount of the formed coloring matter. Copigmentation and self-association of molecules reduce the number of hydroxyl groups. Additionally, other instable substances such as vitamin C, which contains hydroxyl groups, react with the Folin reagent (*35*, *36*). Thus, a decreased content of total phenolics was measured. High losses of anthocyanins were found with 69−79% in both wines after pasteurization, which is in contrast to the losses of phenolics of approximately 37 and 53%. During the fermentation, condensation reactions of anthocyanins were followed by polymerizations to complex and antioxidative active phenolic substances (*9*, *33*). The more the ethanol concentration is increased during the fermentation, the less the extent of copigmentation and self-association is. Therefore, the influence of heat treatment leads to dissociation of the complexes and a release of the flavylium cation that is hydrated to the colorless and labile hemiacetal base, causing a decrease of total anthocyanin content (*33*, *37*).

Table 3. Pearson Correlation ($p = 0.01$) of the Measured Parameters during the Production of Strawberry Juice

strawberry juice	FRAP	TEAC	vitamin C	total phenolics
TEAC	0.979			
vitamin C	0.971	0.980		
total phenolics	0.987	0.977	0.966	
total anthocyanins	0.913	0.930	0.938	0.903

The hydrophilic antioxidant capacity was analyzed by using two methods—FRAP and the TEAC assay—showing different sensitivity and different reaction principles. The measured hydrophilic antioxidant capacity values of 1190 ± 30 μmol of TE/100 g (TEAC assay) and 2466 ± 97 μmol of Fe^{2+}/100 g of fresh strawberries (FRAP assay) (**Table 1**) obtained in this study are comparable to existing data from Olsson et al. (430−900 μmol of TE/100 g of fm) (*22*) and Viberg et al. (*38*), although there are certain differences dependent on species and growing conditions (*12*, *39*−*42*). The decrease of the contents of vitamin C, phenolic compounds, and total anthocyanins led to a reduction of the hydrophilic antioxidant capacity during the processing of strawberries. All these parameters are well-correlated (**Table 3**). Also, the antioxidant capacity could have been influenced by antioxidative Maillard products (*43*), which are formed during pasteurization of juice, nectar, and wine.

Concluding our investigations, nectar, juice, puree, and wine made of strawberries offer an important opportunity to create a healthy, seasonally independent and mixed diet. These products are excellent sources of nutritional substances with antioxidant potential (*13*, *44*−*46*). Although wines have a high hydrophilic antioxidant capacity, one is advised to choose the nonalcoholic products because they offer a higher content of anthocyanins. The puree, produced with only a few processing steps without pasteurization, showed the best qualities of the measured compounds. The amounts of important antioxidative substances decreased with the increase of processing steps and use of heating (**Figure 3**). Summarizing, the experiments led to new results in the field of antioxidant substances in strawberries and their products, especially considering pomace and suspended particles as well as the processing of different products made from identical raw material. Further investigations might assist in determining contents of anthocyanins using, for example, HPLC with its ability to separate anthocyanins and in proving if the hot filling without further pasteurization steps might be a milder method than the cold filling that was used in the processing of juice, nectar, and wine in this study.

ACKNOWLEDGMENT

We thank E. Kettern, Lemgo, Germany, and I. Schmuck as well as H. Schmidt, both of Jena, Germany, for their skillful technical assistance.

LITERATURE CITED

(1) Van Poppel, G.; Kardinal, A.; Princen, H.; Kok, F. J. Antioxidants and coronary heart disease. *Ann. Med.* **1994**, *26*, 426−434.

(2) Rice-Evans, C.; Miller, N. J. Antioxidants—the case for fruit and vegetables in the diet. *Br. Food J.* **1995**, *97*, 35−40.

(3) Steinmetz, K. A.; Potter, J. D. Vegetables, fruit, and cancer prevention: A review. *J. Am. Diet. Assoc.* **1996**, *96*, 1027−1039.

(4) Ames, B. M. Dietary carcinogens and anticarcinogens: oxygen radicals and degenerative diseases. *Science* **1983**, *221*, 1256−1263.

(5) Gey, K. F. The antioxidant hypothesis of cardiovascular disease: epidemiology and mechanisms. *Biochem. Soc. Trans.* **1990**, *18*, 1041−1045.

(6) Steinberg, D. Antioxidants and atherosclerosis: a current assessment. *Circulation* **1991**, *84*, 1420−1425.

(7) Steinberg, D.; Parthasarathy, S.; Carew, T. E.; Khoo, J. C.; Witztum, J. L. Beyond cholesterol: modification of low-density lipoprotein that increase its atherogenicity. *New Engl. J. Med.* **1989**, *320*, 915−924.

(8) Herrmann, K. *Inhaltsstoffe von Obst und Gemüse*; Eugen Ulmer Verlag GmbH and Co.: Stuttgart, 2001.

(9) Rechner, A. Einfluss der Verarbeitungstechnik auf die Polyphenole und antioxidative Kapazität von Apfel- und Beerenobstsäften. Ph.D. Thesis, Justus-Liebig-University Giessen, 2000.

(10) Nielsen, I. L. F.; Haren, G. R.; Magnussen, E. L.; Dragsted, L. O.; Rasmussen, S. E. Quantification of anthocyanins in commercial black currant juices by simple high-performance liquid chromatography. Investigation of their pH stability and antioxidative potency. *J. Agric. Food Chem.* **2003**, *51*, 5861−5866.

(11) Lapidot, T.; Harel, S.; Akiri, B.; Granit, R.; Kanner, J. pH-dependent forms of red wine anthocyanins as antioxidants. *J. Agric. Food Chem.* **1999**, *47*, 67−70.

(12) Proteggente, A. R.; Pannala, A. S.; Paganga, G.; van Buren, L.; Wagner, E.; Wiseman, S.; van De Put, F.; Dacombe, C.; Rice-Evans, C. A. The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radical Res.* **2002**, *36*, 217−233.

(13) Böhm, V. Bestimmung der antioxidativen Kapazität—methodische Ansätze und Bewertung. *Ernährungs-Umschau* **2000**, *47*, 372−375.

(14) Silbereisen, R.; Götz, G.; Hartmann, W. *Obstsorten-Atlas Kernobst, Steinobst, Beerenobst, Schalenobst. 2. Auflage*; Eugen Ulmer Verlag GmbH and Co.: Stuttgart, 1996.

(15) Speitling, A.; Hüppe, R.; Kohlmeier, M.; Matiaske, B.; Stelte, W.; Thefeld, W.; Wetzel, S. Methodological Handbook Nutrition Survey and Risk Factors Analysis. In *Vera Publication Series*; Kübler, W., Anders, H. J., Heeschen, W., Kohlmeier, M., Eds.; Wissenschaftlicher Fachverlag Dr. Fleck: Niederkleen, Germany, 1992; Vol IA, pp 103−105.

(16) Vazquez Oderiz, M. E.; Vazquez Blanco, M. E.; Lopez Hernandez, J.; Simal Lozano, J.; Romero Rodriguez, M. A. Simultaneous determination of organic acids and vitamin C in green beans by liquid chromatography. *J. AOAC Int.* **1994**, *77*, 1056−1059.

(17) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. *Am. J. Enol. Vitic.* **1965**, *37*, 144−158.

(18) Gao, L.; Mazza, G. Characterization, quantitation, and distribution of anthocyanins and colorless phenolics in sweet cherries. *J. Agric. Food Chem.* **1995**, *43*, 343−346.

(19) Cheng, G. W.; Breen, P. J. Activity of phenylalanine ammonia-lyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. *J. Am. Soc. Hortic. Sci.* **1991**, *116*, 865−869.

(20) Schlesier, K.; Harwat, M.; Böhm, V.; Bitsch, R. Assessment of antioxidant activity by using different in vitro methods. *Free Radical Res.* **2002**, *36*, 177−187.

(21) Böhm, V.; Schlesier, K. Methods to evaluate the antioxidant activity. In *Quality Handling and Evaluation, Production Practices and Quality Assessment of Food Crops*; Dris, R., Jain, S. M., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2004; Vol. 3, pp 55−71.

(22) Olsson, M. E.; Ekvall, J.; Gustavsson, K.-E.; Nilsson, J.; Pillai, D.; Sjöholm, I.; Svensson, U.; Akesson, B.; Nyman, M. G. Antioxidants, low molecular weight carbohydrates, and total antioxidant capacity in strawberries (Fragaria × ananassa): effects of cultivar, ripening, and storage. *J. Agric. Food Chem.* **2004**, *52*, 2490−2498.

(23) Heinonen, I. M.; Meyer, A. S.; Frankel, E. N. Antioxidant activity of berry phenolics on human low-density lipoprotein and liposome oxidation. *J. Agric. Food Chem.* **1998**, *46*, 4107−4112.

(24) Meyers, K. J.; Watkins, C. B.; Pritts, M. P.; Liu, R. H. Antioxidant and antiproliferative activities of strawberries. *J. Agric. Food Chem.* **2003**, *51*, 6887−6892.
(25) Velioglu, Y. S.; Mazza, G.; Gao, L.; Oomah, B. D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J. Agric. Food Chem.* **1998**, *46*, 4113−4117.
(26) Wang, S. Y.; Lin, H.-S. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *J. Agric. Food Chem.* **2000**, *48*, 140−146.
(27) Clifford, M. N. Anthocyanins—nature, occurrence, and dietary burden. *J. Sci. Food Agric.* **2000**, *80*, 1063−1072.
(28) Macheix, J.-J.; Fleurit, A.; Billot, J. *Fruit phenolics*; CRC Press: Boca Raton, FL, 1990.
(29) Gil, M. I.; Holcroft, D. M.; Kader, A. A. Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. *J. Agric. Food Chem.* **1997**, *45*, 1662−1667.
(30) Asen, S.; Steward, R. N.; Norris, K. H. Copigmentation of anthocyanins in plant tissues and its effect on color. *Phytochemistry* **1972**, *11*, 1139−1144.
(31) Hoshino, T. An approximate estimate of self-association constants and self-stacking confirmation of malvin quinoidal bases studied by ^{1}H NMR. *Phytochemistry* **1991**, *30*, 2049−2055.
(32) Hoschino, T. Self-association of flavylium cations of anthocyanidin 3,5-diglucosides studied by circular dichromism and ^{1}H NMR. *Phytochemistry* **1992**, *31*, 647−653.
(33) Parley, A. The effect of prefermentation enzyme maceration on extraction and color stability in Pinot noir wine. M.S. Thesis, Lincoln University, Canterbury, 1997.
(34) Sarni, P.; Fulcrand, H.; Souillol, V.; Souguet, J.-M.; Cheynier, V. Mechanism of anthocyanin degradation in grape must-like model solutions. *J. Sci. Food Agric.* **1995**, *69*, 385−391.
(35) Ikawa, M.; Schaper, T. D.; Dollard, C. A.; Sasner, J. J. Utilization of Folin−Ciocalteu phenol reagent for the detection of certain nitrogen compounds. *J. Agric. Food Chem.* **2003**, *51*, 1811−1815.
(36) Vinson, J. A.; Proch, J.; Bose, P. Determination of quantity and quality of polyphenol antioxidants in food and beverages. *Methods Enzymol.* **2001**, *335*, 103−107.
(37) Dangles, O.; Brouillard, R. Polyphenol interactions. The copigmentation case: thermodynamic data from temperature variation and relaxation kinetics. Medium effect. *Can. J. Chem.* **1992**, *70*, 2174−2189.
(38) Viberg, U. Studies of the effects of industrial processing of fruit. Ph.D. Thesis, Lund Institute of Technology, Lund, Sweden, 1998.
(39) Guo, C.; Yang, J.; Wei, J.; Li, Y.; Xu, J.; Jiang, Y. Antioxidant activities of peel, pulp, and seed fractions of common fruits as determined by FRAP assay. *Nutr. Res.* **2003**, *23*, 1719−1726.
(40) Halvorsen, B. L.; Holte, K.; Myhrstad, M. C. W.; Barikmo, I.; Hvattum, E.; Fagertun Remberg, S.; Wold, A.-B.; Haffner, K.; Baugerod, H.; Frost Andersen, L.; Moskaug, J. O.; Jacobs, D. R.; Blomhoff, R. A systematic screening of total antioxidants in dietary plants. *J. Nutr.* **2002**, *132*, 461−471.
(41) Leong, L. P.; Shui, G. An investigation of antioxidant capacity of fruits in Singapore markets. *Food Chem.* **2002**, *76*, 69−75.
(42) Wang, H.; Cao, G.; Prior, R. L. Total antioxidant capacity of fruits. *J. Agric. Food Chem.* **1996**, *44*, 701−705.
(43) Lingnert, H.; Waller, G. R. Stability of antioxidants formed from histidine and glucose by the Maillard reaction. *J. Agric. Food Chem.* **1983**, *31*, 27−30.
(44) Gardner, P. T.; White, T. A. C.; McPhail, D. B.; Duthie, G. G. The relative contributions of vitamin C, carotenoids, and phenolics to the antioxidant potential of fruit juices. *Food Chem.* **2000**, *68*, 471−474.
(45) Scalbert, A.; Williamson, G. Dietary Intake and Biovailability of polyphenols. *J. Nutr.* **2000**, *130*, 2073S−2085S.
(46) Henn, T.; Stehle, P. Gesamtphenolgehalt und antioxidative Kapazität handelsüblicher Getränke. *Ernährungs-Umschau* **1998**, *45*, 308−313.

Received for review December 6, 2004. Revised manuscript received May 3, 2005. Accepted May 10, 2005.

JF047947V