Influence of Processing on Quality Parameters of Strawberries

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To determine the effects of different processing steps, such as enzymatic treatment of the mash and pasteurization, on selected quality parameters, strawberries were processed to juices and purees. To identify the processing steps causing the highest losses, samples were taken after each step, and ascorbic acid, total phenols, anthocyanins, and antioxidant capacity were analyzed. To assess the antioxidant capacity, three different methods were applied: the trolox equivalent antioxidant capacity (TEAC), the ferric reducing antioxidant power (FRAP), and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, showing correlation coefficients of 0.889 to 0.948. The antioxidant capacity decreased with processing steps except heat treatment, which partly caused an increase due to the formation of antioxidant active products. The content of ascorbic acid, in comparison to that in the frozen strawberries, decreased significantly during the processing of the fruit to puree by 77%. In the pressed cloudy juices, the loss of ascorbic acid was 37%. The decline of phenolic compounds, measured as total polyphenols and anthocyanins, was smaller (between 30−40%). Pressing and pasteurization were the most critical steps for the decrease of these compounds. The enzymatic treatment of the mash within 90 min supported the release of secondary plant metabolites, while ascorbic acid is reduced up to 20%.

INTRODUCTION

Experimental and epidemiological studies show a clear correlation between high intakes of fruits and vegetables and the prevention or delay of coronary heart diseases and certain forms of cancer (*1−7*). This preventive action is mainly ascribed to phytochemicals possessing antioxidant properties such as L-ascorbic acid, vitamin E, carotenoids, and polyphenols (*5*).

The daily intake of five portions of fruit and vegetables is recommended in many countries. These guidelines are not achievable for many people due to the required variety of fruit and vegetables among others. Thus, the replacement of one or several of these portions by fruit juices, concentrates, or smashed fruits (smoothies) is suggested.

Antioxidant capacity and phenolic content of fruit juices mainly depend on the fruit itself, but processing and storage conditions are also important influential factors (*8−15*). The antioxidant capacity varies highly between different plants, but cultivar, condition of cultivation, and ripeness are also important factors (*16−18*). The antioxidant activity of juices can therefore be enhanced through the selection of adequate fruits.

Strawberries show a large spectrum of phenolic components in which not only the colored anthocyanins, but also the colorless phenols (particularly ellagic acid, ellagitannins, *p*-coumaric acid, and quercetins) contribute to the richness of secondary plant metabolites (*19*).

Even though the positive effects of fruits on human health are well-known, flavor and color are most important factors for consumers for the selection of juices.

The colored anthocyanins are subjected to intense changes during processing and storage. Stability depends on their structure as well as on the matrix and composition of the medium. Temperature, pH, light, oxygen and ascorbic acid highly affect the half-life of anthocyanins (*20*). A considerable degradation of anthocyanins in processed strawberries is due to oxidative enzymes (*21*). Anthocyanins also condense with other phenolic compounds to form oligo- and polymers. Therefore, noticeable changes of anthocyanins during processing and storage are visible to the naked eye, especially in the case of strawberry products which are characterized by fast browning.

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Quality Parameters of Strawberries

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To best preserve the valuable substances of fruits during processing, it is important to identify the processing steps which cause the highest losses. Quantification of these losses enables the consideration of optimization possibilities as well as the quest of processing techniques, manner of storage, and raw materials to reduce these losses.

In the present study, the influence of main processing steps on quality parameters of strawberry juice and strawberry puree was investigated. For the juices and the purees, the effect of mash enzymation on the release of secondary plant metabolites are compared.

MATERIALS AND METHODS

Reagents and Standards. Folin−Ciocalteu's phenol reagent, potassium persulfate, dipotassium hydrogenphosphate, sulfuric acid (98%), methanol (p.a.), and acetone (p.a.) were from VWR; L(+)-ascorbic acid, oxalic acid, hydrochloric acid, sodium acetate, and sodium−carbonate monohydrate were from Carl Roth GmbH; and Ferric(III)-chloride hexahydrate was from Riedel-de Haën. 1/128 mol/L potassiumiodate/ potassiumiodide-solution was from Bernd Kraft GmbH; 2,2′-azino-bis- (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2- diphenyl-1-picrylhydrazyle (DPPH), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), gallic acid monohydrate (3,4,5- trihydroxybenzoic acid), and 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ) were from Fluka.

Sample Preparation. Purees and juices were produced from frozen strawberries (*Fragaria × ananassa* var. Senga sengana) according to the process described below. Samples were taken after each processing step and after three, seven, and eleven weeks of storage at 8 °C. All these samples and the frozen strawberries were stored at −28 °C until analysis. For the analysis, the samples where thawed in a water bath at 40 °C for 30 min. Nonliquid samples (fruits, purees) were homogenized before analysis. The extract was prepared by a method described by Scalzo et al. (*16*) and Gao and Mazza (*22*). Thirty grams of the homogenized sample or juice was extracted with 50 mL of methanol/ H_2O/acetone (60 + 30 + 10; v/v/v). The homogenized extract was centrifuged, and the clear phase was separated and filled to 100 mL with the extraction mixture. This extract was used for the determination of the antioxiative capacity, the polyphenols, and the anthocyanins. Three replicates were done for each determination.

Ascorbic Acid. The ascorbic acid content was determined by iodometric titration. The sample was homogenized with oxalic acid (2%, w/v) (1 + 3), acidified with sulfuric acid (10%), and titrated with a 1/128 mol/L iodide-iodate-solution with a double platinum electrode.

Anthocyanins. The procedure described by Giusti and Wrolstad was followed (*23*). Anthocyanins were calculated as Pelargonidin 3-*O*- glucoside (Pg-3-glu). The sample was extracted with a solution of methanol/acetone/water (60 + 30 + 10; v/v/v). The extract was diluted with two buffer solutions at pH 1 and 4.5. The absorbance was measured at 500 and 700 nm, and the results were calculated according to the following equation and given in mg pelargonidin-3-glucoside per kg of fresh weight.

$$Pg\text{-}3\text{-}glu[mg/kg] = \frac{[(A_{500} - A_{700})_{pH1.0} - (A_{500} - A_{700})_{pH4.5}] \times MW \times DF \times V \times 10^6}{(\varepsilon \times W)}$$

In the above equation A is absorption; MW is molecular weight of Pg- 3-glu (433.2 g/mol); DF is dilution factor; V is volume of the extract in L; molar absorption coefficient ε of Pg-3-glu 15600 L/(mol × cm); and W is sample weight in g.

Total Polyphenols. Total polyphenols were determined by the Folin−Ciocalteu method (*24, 25*). One milliliter of the extract was mixed with 7.5 mL of the Folin−Ciocalteu reagent and allowed to stand for 6 min. Then 1 mL of a saturated sodium−carbonate solution was added and mixed. After 60 min, the absorbance was measured at 720 nm. Gallic acid monohydrate was used as reference compound, and the total polyphenol content is expressed in mg of gallic acid equivalents/kg of fresh weight.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay. A slightly modified method of Alamanni and Cossu (*26*) was used in which a stable radical (DPPH) is reduced by the antioxidants of the sample, which leads to a decolorization. Ten milliliters of a 0.1 mmol/L DPPH solution in methanol were mixed with 0.1 mL sample extract. After 30 min, the absorbance was measured at 517 nm. Trolox was used as reference compound, and the antioxidant capacity is expressed in mmol/L trolox equivalents per kg of fresh weight.

Ferric Reducing Antioxidant Power (FRAP) Assay. The method described by Benzie and Strain (*27*) modified by Guo et al. (*28*) was followed, which is based on the reduction of a ferric(III) complex to its colored ferrous(II) form in the presence of antioxidants. The FRAP reagent was prepared freshly before measuring and contained 5 mL of a 10 mmol/L TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) solution in 40 mmol/L hydrochloric acid, 5 mL of a 20 mmol/L ferric(III)chloride solution, and 50 mL of 0.3 mol/L acetate buffer (pH 3.6). The extract (0.1 mL) was mixed with 0.3 mL of water and 3 mL of reagent. After 8 min, the absorbance was measured at 593 nm. Trolox was used as the reference compound, and the antioxidant capacity is expressed in mmol trolox equivalents/kg of fresh weight.

Trolox Equivalent Antioxidant Capacity (TEAC) Assay. A slightly modified method of Re et al. (*29*) was followed, which is based on the generation of a durable $ABTS^{\bullet+}$ radical solution. This solution is reduced by the antioxidant molecules of the sample, which leads to a decolorization of the solution. 2.45 mmol/L potassium persulphate was added to a 7 mmol/L ABTS solution in 50 mmol/L phosphate buffer (pH 7.3). The solution was allowed to stand in the dark at room temperature for at least 6 h. Before measuring, the ABTS solution was diluted with phosphate buffer in such a manner that the absorbance was situated between 0.700 and 0.900. This solution (1.9 mL) was mixed with 0.1 mL of the extract, and after 6 min, the absorbance was measured at 734 nm. Trolox was used as a reference compound, and the antioxidant capacity is expressed in mmol/L trolox equivalents/kg of fresh weight.

Statistical Analysis. All samples were analyzed statistically with the *F*- and *t* test to determine possible significant differences. Data are reported as a mean ± confidence interval of 95% of three measurements of every sample ($n = 3$). All indicated values apply to the fresh weight of the strawberries.

Production of Strawberry Juice. The strawberries (40.5 kg) were thawed overnight (15 h) at 19 °C. The cold strawberries (0 °C, juice sample 2) were crushed with a roller mill within 5 min (Defranceschi Advanced Beverage Equipment Worldwide, model ABB). The mash was heated in a water bath up to 25 °C (juice sample 3). The mash (38 kg) was divided into two parts. Part one (18.7 kg) was heated up to 45 °C and was treated with Fructozym Color (Erbsloeh 100 mL/1000 kg) for 90 min (juice sample 7). Part two (17.7 kg) was pressed immediately without mash enzymation with a rack and cloth press (Wahler, model HPP 400) within 1 h (50 bar, juice 13.8 kg, pomace 3.4 kg, juice sample 4). One part of the pressed juice (6.1 kg) was filled into 330 mL glass bottles and pasteurized at 85 °C for 15 min in a water bath (juice sample 6), and the other part (6.1 kg) was hot-filled (85 °C, 5 s) into 330 mL PET bottles (juice sample 5). For all hot fillings in PET, a flow pasteurization unit from Mabo-Fruchtsaftdispenser (Mabo, Germany) was used.

The enzyme-treated mash was pressed with the rack and cloth press within 15 min (juice 15.3 kg, pomace 1.3 kg, juice sample 8). The pressed juice was hot-filled (Mabo, 85 °C, 5 s) into 330 mL PET bottles (juice sample 9).

During all processing steps, samples between 1 and 1.6 kg were taken and stored frozen in smaller portions at −28 °C until analysis.

Production of Strawberry Puree. The strawberries (16.3 kg) were thawed overnight (15 h) at 19 °C. The cold strawberries (15 kg at −1 °C) were heated in a water bath up to 25 °C within 60 min (puree sample 2). After this, the strawberries were crushed by hand with a masher (puree sample 3). The mash was divided into two parts. Part one (7 kg) was enzymatically treated with Vegazym M (Erbsloeh, a maceration enzyme, 225 mL/1000 kg,) during 90 min at 25 °C (puree sample 6). The mash was finished through a stainless steel sieve (mash diameter ~1 mm) to get a puree (puree sample 7). The sieve residuum from the enzymatic treated mash was below 0.1% (w/w), from the

Table 1. Change of Ascorbic Acid, Total Polyphenols, Anthocyanins, and Antioxidant Capacity during the Processing of Strawberries to Juices[a]

samples	ascorbic acid	polyphenols	anthocyanins	TEAC	DPPH	FRAP
(1) frozen strawberries (= 100%)	378 ± 2 a [mg/kg]	2267 ± 26 a [mg/kg]	508 ± 5 a [mg/kg]	23.98 ± 0.79 a [mmol/kg]	9.70 ± 0.56 a [mmol/kg]	9.73 ± 0.41 a [mmol/kg]
(2) mash at 0 °C [%]	78.2 ± 0.8 b	99.3 ± 0.8 ab	94.7 ± 1.2 b	97.2 ± 1.5 b	97.7 ± 3.7 ab	99.4 ± 3.4 a
(3) mash at 25 °C [%]	75.4 ± 2.7 b	98.8 ± 1.1 b	93.7 ± 1.0 c	96.7 ± 1.5 b	94.1 ± 6.2 bf	99.9 ± 4.4 a
(4) juice [%]	76.8 ± 1.6 b	69.3 ± 2.0 c	77.8 ± 1.8 d	72.4 ± 0.0 c	63.5 ± 3.9 c	73.6 ± 2.5 b
(4) pomace [%]	41.3 ± 6.4	233.8 ± 2.6	288.2 ± 1.0	248.1 ± 9.0	227.3 ± 10.5	223.1 ± 9.0
(5) past. juice (PET) [%]	63.5 ± 0.8 c	64.9 ± 0.7 d	70.7 ± 0.6 e	71.8 ± 1.8 c	69.7 ± 2.3 d	73.7 ± 2.5 b
(6) bottle past. juice (glass) [%]	46.1 ± 1.9 d	62.3 ± 0.9 e	61.4 ± 0.8 f	71.1 ± 5.3 c	67.1 ± 1.6 e	73.5 ± 4.3 b
(7) enz. mash [%]	53.7 ± 2.4 e	98.4 ± 1.3 b	89.2 ± 0.8 g	95.8 ± 3.3 b	93.8 ± 1.2 f	99.8 ± 6.2 a
(8) enz. juice [%]	56.1 ± 0.5 f	79.7 ± 2.6 f	87.0 ± 0.8 h	87.1 ± 3.0 d	76.7 ± 2.0 g	87.2 ± 5.8 c
(8) enz. pomace [%]	36.8 ± 0.3	280.5 ± 2.2	221.1 ± 2.0	278.4 ± 6.6	230.8 ± 2.8	270.8 ± 12.6
(9) past. enz. juice (PET) [%]	39.4 ± 1.6 g	78.3 ± 0.6 f	76.2 ± 1.0 i	87.7 ± 3.5 d	78.5 ± 2.0 gh	88.0 ± 4.4 c

[a] Results for frozen strawberries are given in absolute values. All other values are relative (%) with reference to frozen strawberries (100%). Values with different letters within one column are significantly different (α = 0.05). The numbers in parentheses represent the processing stage described in the text.

Table 2. Change of Ascorbic Acid, Total Polyphenols, Anthocyanins, and Antioxidant Capacity during the Storage of Juices at 8 °C[a]

frozen strawberries = 100%	ascorbic acid [%]	polyphenols [%]	anthocyanins [%]	TEAC [%]	DPPH [%]	FRAP [%]
(5) past. juice (PET)	63.5 ± 0.8 a	64.9 ± 0.7 a	70.7 ± 0.6 a	71.8 ± 1.8 a	69.7 ± 2.3 a	73.7 ± 2.5 a
(6) bottle past. juice (glass)	46.1 ± 1.9 b	62.3 ± 0.9 b	61.4 ± 0.8 b	71.1 ± 5.3 ad	67.1 ± 1.6 b	73.5 ± 4.3 a
(9) past. enz. juice (PET)	39.4 ± 1.6 c	78.3 ± 0.6 c	76.2 ± 1.0 c	87.7 ± 3.5 b	78.5 ± 2.0 c	88.0 ± 4.4 b
			After 3 Weeks of Storage			
(5) past. juice (PET)	51.4 ± 1.1 e	64.5 ± 0.6 ah	62.8 ± 0.8 e	71.4 ± 4.5 ad	66.0 ± 3.5 b	72.5 ± 9.0 ac
(6) bottle past. juice (glass)	28.6 ± 2.7 f	60.2 ± 1.4 ej	49.0 ± 0.2 f	69.8 ± 2.7 ad	67.3 ± 2.1 b	67.1 ± 3.1 ac
(9) past. enz. juice (PET)	32.8 ± 2.7 g	76.1 ± 2.1 f	65.4 ± 1.2 g	86.8 ± 2.5 b	78.9 ± 1.9 c	78.6 ± 10 bd
			After 7 Weeks of Storage			
(5) past. juice (PET)	49.3 ± 1.3 i	63.5 ± 2.1 bhi	54.1 ± 0.8 i	70.6 ± 2.5 ad	69.9 ± 1.7 a	69.7 ± 3.8 c
(6) bottle past. juice (glass)	23.6 ± 3.2 d	61.0 ± 1.2 e	41.9 ± 0.6 j	69.4 ± 3.3 d	67.2 ± 2.4 b	67.0 ± 2.1 c
(9) past. enz. juice (PET)	28.1 ± 1.6 f	72.6 ± 1.4 d	51.8 ± 0.8 k	79.6 ± 2.5 c	75.7 ± 1.8 e	75.4 ± 2.0 e
			After 11 Weeks of Storage			
(5) past. juice (PET)	40.8 ± 3.2 c	63.7 ± 1.0 i	46.5 ± 0.8 m	70.1 ± 2.5 ad	69.0 ± 3.6 ab	68.8 ± 5.3 ac
(6) bottle past. juice (glass)	15.6 ± 2.7 hj	58.9 ± 1.8 j	35.8 ± 0.8 n	68.3 ± 4.5 d	66.3 ± 2.1 b	66.1 ± 3.8 g
(9) past. enz. juice (PET)	26.5 ± 2.7 f	72.4 ± 1.4 d	46.3 ± 0.8 m	79.7 ± 4.4 c	78.9 ± 1.9 c	78.6 ± 4.6 def

[a] Results are given in relative values (%) with reference to frozen strawberries (100%). Values with different letters within one column are significantly different (α = 0.05). The numbers in parentheses represent the processing stage described in the text.

untreated part ∼4% from the sieved mash. The finished puree was heated in a water bath and kept for 2 min at 85 °C before hot-filled into 375 mL preserving glasses (puree sample 8).

Part two was finished directly without holding time after crushing through a stainless steel sieve (puree sample 4), heated, and hot-filled in preserving glasses (puree sample 5) as described above.

Samples were taken after the thawing (1.3 kg) and mashing (1.3 kg); part one, after enzymatic treatment (1.3 kg) and after passing through a sieve (1.3 kg); part two after passing through a sieve (1.4 kg).

Storage of Juice and Puree. To simulate the effect of storage on antioxidants, the pasteurized cloudy portion and the purees were stored at 8 °C; samples were taken at 3, 7, and 11 weeks.

RESULTS

Ascorbic Acid. As shown in **Table 1**, the ascorbic acid content decreased continuously during the processing of cloudy juices as well as during subsequent storage (**Table 2**). The highest losses were caused by thawing (22% diminution), enzymatic mash treatment within 90 min, and pasteurization. In the case of the freshly pressed and cloudy strawberry juice, 76.8% of the original ascorbic acid was preserved (no. 4); a smaller portion remained in the pomace. Flash pasteurization and filling into PET bottles (no. 5) reduced the ascorbic acid from 76.8% to 63.5% of the original frozen strawberries. This corresponds to a loss of 17.4% by the pasteurization step (compare nos. 5 and 4). The bottle pasteurization for 15 min led to a strong decline of ascorbic acid (compare no. 6 and no. 4); therefore, this technique is no longer used in the industry.

The final cloudy juice retained 63.5% of the original concentration of frozen strawberries.

Table 2 shows the effect of storage at 8 °C for 11 weeks. In the cloudy strawberry juice (juice sample 5), which had 63.5% ascorbic acid before storage, ascorbic acid decreased to 40.8% compared to frozen strawberries (100%). This corresponds to a loss of 35.7% during storage.

Table 3 shows the influence of puree processing with and without enzymatic maceration and subsequent storage on the antioxidants of strawberry purees. In the puree before pasteurization (puree sample 4), 48.4% of the original ascorbic acid was retained, which is worse than it was with pressed juice. The enzymatic maceration did not have much influence (compare puree sample 7 and 4), whereas the subsequent pasteurization into bottles (flash pasteurization at 85 °C for 2 min) had a pronounced effect on enzymated and nonenzymated purees (puree sample 5 and 8). About 23% ascorbic acid was retained in both products. Subsequent storage led to a further decrease (**Table 3**).

Total Polyphenols. Polyphenols and anthocyanins were more stable than ascorbic acid during processing and storage of the juices (**Table 1**). Juice extraction without enzymatic treatment of the mash was the processing step involving the biggest loss of polyphenols (30.7%, juice sample 4). The enzymatic treatment before juice extraction led to a loss of only 20.3%. Enzymatic mash treatment seemed to ameliorate the release of polyphenols. Pasteurization led to a decrease of about 10% in the juices (**Table 1**), which were heated in bottles, and 6% when pasteurized for 5 s. Storage of the juices at 8 °C for 11 weeks

Table 3. Values of Ascorbic Acid, Total Polyphenols, Anthocyanins, and Antioxidant Capacity during the Processing of Strawberries to Puree and Storage at 8 °C[a]

samples	ascorbic acid	polyphenols	anthocyanins	TEAC	DPPH	FRAP
(1) frozen strawberries (= 100%)	386 ± 14 a [mg/kg]	2261 ± 14 a [mg/kg]	510 ± 6 a [mg/kg]	23.87 ± 0.5 a [mmol/kg]	9.65 ± 0.1 a [mmol/kg]	9.78 ± 0.4 a [mmol/kg]
(2) strawberries at 25 °C [%]	66.1 ± 3.4 b	98.9 ± 1.1 b	97.4 ± 2.0 b	99.6 ± 5.0 ab	96.6 ± 3.6 b	97.9 ± 1.2 a
(3) mash [%]	49.8 ± 3.4 c	98.7 ± 1.4 bc	95.7 ± 2.0 b	99.3 ± 4.4 ab	96.6 ± 1.5 b	99.3 ± 2.7 a
(4) puree [%]	48.4 ± 2.3 c	97.6 ± 1.4 c	90.1 ± 1.8 ce	98.0 ± 2.1 bc	94.3 ± 2.4 c	98.6 ± 2.4 a
(5) pasteurized puree [%]	23.3 ± 1.6 d	86.7 ± 1.6 d	76.2 ± 0.8 d	95.5 ± 3.6 cd	84.6 ± 2.1 d	91.5 ± 3.2 b
(6) enz. mash [%]	47.5 ± 3.6 ce	98.2 ± 1.0 bc	90.9 ± 0.6 c	97.8 ± 4.2 acd	91.0 ± 3.5 c	98.1 ± 6.0 ac
(7) enz. puree [%]	45.9 ± 2.9 e	96.5 ± 0.8 e	89.9 ± 0.6 e	95.3 ± 1.2 d	85.8 ± 2.0 ed	95.2 ± 2.2 c
(8) past. enz. puree [%]	23.1 ± 1.6 d	81.6 ± 1.6 f	68.1 ± 0.8 f	89.8 ± 2.4 e	86.4 ± 1.6 e	89.1 ± 7.0 bd
(5) past. puree, stored 3 weeks [%]	16.2 ± 2.3 f	77.4 ± 1.6 gj	56.0 ± 0.4 g	85.1 ± 2.7 fgh	83.1 ± 1.5 df	88.7 ± 6.3 bd
(8) past. enz. puree, stored 3 weeks [%]	15.7 ± 2.6 f	75.1 ± 0.4 h	56.8 ± 1.8 g	82.7 ± 3.5 gh	81.7 ± 2.0 fh	85.4 ± 5.7 de
(5) past. puree, stored 7 weeks [%]	12.2 ± 1.8 g	79.7 ± 1.3 ij	48.1 ± 0.8 h	86.8 ± 2.7 f	77.3 ± 2.3 g	81.9 ± 5.1 e
(8) past. enz. puree, stored 7 weeks [%]	10.4 ± 2.6 g	79.4 ± 0.8 ij	51.5 ± 1.2 i	85.0 ± 1.2 hf	76.4 ± 3.2 g	82.2 ± 6.2 e
(5) past. puree, stored 11 weeks [%]	7.8 ± 1.6 h	79.7 ± 2.2 ij	41.3 ± 0.8 j	85.6 ± 2.2 f	79.3 ± 4.8 hg	82.6 ± 4.1 e
(8) past. enz. puree, stored 11 weeks [%]	10.9 ± 1.8 g	78.6 ± 1.6 j	43.1 ± 1.0 k	84.2 ± 3.8 hf	77.7 ± 3.6 g	80.6 ± 4.6 e

[a] Results for frozen strawberries are given in absolute values. All other values are relative (%) with reference to frozen strawberries (100%). Values with different letters within one column are significantly different (α = 0.05). The numbers in parentheses represent the processing stage described in the text.

caused a decrease of polyphenols in the range of 2−8% as shown in **Table 2**.

Table 3 shows that the polyphenols are better retained in the purees than in the juices. In comparison to frozen strawberries, the puree contained 97.6% (puree sample 4) and the enzymated puree 96.5% (puree sample 7) of the polyphenols. After pasteurization, 86.7% and 81.6%, respectively, of the polyphenols were retained. The subsequent loss of polyphenols during storage was rather small (**Table 3**). After 11 weeks at 8 °C, 79.7% and 78.6% of the polyphenols were retained in the nonenzymated and enzymated purees.

Anthocyanins. The steps with the highest influence on the anthocyanin content were juice extraction and pasteurization (**Tables 1** and **3**). The juice from the nonenzymated mash contained 77.8% (juice sample 4, **Table 1**) of the frozen berries and the juice made from enzymated mash 87% (juice sample 8). This means that part of the anthocyanins is lost in the corresponding pomaces. Enzymatic mash treatment seems to be favorable for the release of anthocyanins as it leads to greater recovery of pigments. A severe loss of anthocyanins was caused by pasteurization in bottles for 15 min (21% diminution, compare juice sample 4 and 6), while a short heating time for 5 s before filling caused milder losses of 9−14%. Pasteurization of the puree led to a decrease of 15.5% and 24.2% in the nonenzymated and enzymated puree (**Table 3**).

A storage at 8 °C for 11 weeks also caused extensive losses of an average of 39% (**Table 2**). Almost every processing step caused a significant loss of the anthocyanin content, which emphasizes the high sensitivity of these substances to oxidation, light, and heat.

Antioxidant Capacity. Data from all three applied methods showed similar decreases of the antioxidant capacity during processing. However, there were noticeable differences related to pasteurization (**Tables 1** and **3**). With reference to the purees, DPPH showed the largest decrease of 10% by heat treatment of the puree without enzymatic treatment but displayed no change at all by the pasteurization of the enzymatically treated puree (**Table 3**). The other two methods registered a decrease of 6% by the enzymatic treated puree and showed losses of 7% (FRAP) and 3% (TEAC), respectively, by heat treatment of the nonenzymatically treated puree. As for the juices (**Table 1**), TEAC and FRAP detected no significant differences by heat treatment of the three cloudy juices. DPPH showed a considerable rise of the antioxidant capacity by heating regarding the two enzymatically untreated juices of 6−10%. As polyphenols and anthocyanins were decreasing, it can be assumed that new antioxidant substances are formed during pasteurization.

Losses due to juice extraction could be reduced from 28 to 13% (**Table 1**) through enzymatic treatment of the mash. Storage of juices caused only mild losses of 6−11%. The results of the TEAC, FRAP, and DPPH were slightly different because of the varying sensitivity of these substances to the fruit ingredients.

DISCUSSION

Strawberries contain a large number of secondary plant metabolites. In the variety *Senga sengana*, more than 40 polyphenols were detected among several quercetin derivatives, kaempferol, and flavanols including procyanidins, cinnamic acids, ellagic acid, and ellagitannins; also, a quercetin-3-malonylhexoside and a deoxyhexoside of ellagic acid were described (*11*). A high concentration of ellagitannins, which are said to be antimutagenic and anticarcinogenic, is typical for strawberries. In frozen fruits of *Senga sengana*, 315 mg/kg f.w. ellagic acid derivatives were found (*12*), whereas Thielen (*8*) found 4−24 mg/kg free ellagic acid and total ellagic acids up to 500 mg/kg in fresh fruits. Strawberries contain 1% f.w. achenes, which contribute 11% to the total polyphenol content and 14% to the antioxidative capacity (*30*). Ellagic acid, ellagic acid glycosides, and ellagitannins contribute the most to the antioxidative capacity of the achenes (*30*). Strawberries are also characterized by the presence of anthocyanins, mainly pelargonidin-3-glucoside. Bakker et al. (*31*) found six pelargonidin-derivatives with pelargonidin-3-glucoside as the main compound (82−100%). Also, cyanidins are present in small amounts. Pelargonidin-3-glucoside is found in the fruit flesh, whereas achenes contain similar concentrations of pelargonidin-3-glucoside and cyanidin-3-glucoside (*30*).

On the basis of these facts, the strawberry is a valuable fruit for the fruit juice industry. Nevertheless, the introduction of pure fruit juices, nectars, or fruit based drinks is hindered by the fast aging during processing and storage because of color change, browning, and loss of texture (*12, 14, 32*).

The results clearly indicate that every step during processing of strawberries to purees and juices reduces the content of ascorbic acid, polyphenols, anthocyanins, and antioxidant capacity. The diminution of the total polyphenol content entailed a decrease of the antioxidant capacity. Total polyphenols (Folin−Ciocalteu) and antioxidant capacity (DPPH, FRAP, and TEAC) are well correlated (**Table 4**). However, the extent of the respective losses depends on the analyzed parameter and the processing step.

Thawing Strawberries. Thawing strawberries and fruit mashing showed a negative effect on the ascorbic acid content,

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Table 4. Correlation Coefficients of Measured Values of Total Polyphenols (Folin–Ciocalteu, FC) and Antioxidant Capacity (DPPH, FRAP, and TEAC)

method	TEAC	FC	DPPH
FC	0.9325		
DPPH	0.8885	0.9028	
FRAP	0.9481	0.9262	0.9211

which decreased by about 25%, while the antioxidant capacity, total polyphenols, and anthocyanins were only slightly affected. The maximal loss registered anthocyanins and antioxidant capacity with about 3 to 6%. This highly more pronounced diminution of the ascorbic acid content compared to the other parameters is most probably due to the antioxidant action of ascorbic acid, which protects phenolic substances from oxidation or reduces oxidized polyphenols (quinones).

Enzymatic Mash Treatment. The enzymatic mash treatment supports the release of polyphenols and is necessary for a higher yield of polyphenols and anthocyanins in the pressed juices. However, this treatment in combination with mash standing time diminished the content of ascorbic acid up to 30%. Short enzymation times are recommended. By enzymatic treatment of the mash, a yield of about 72% of the possible phenolic recovery was reached, while the yield without enzymatic treatment was only about 55%. Also, the yield of anthocyanins could be boosted by the pectinase treatment of the mash, which was about 87% compared to about 65% without this treatment. The comparatively high yield of anthocyanins is probably due to the release from solid fruit parts and the good water solubility of anthocyanins, which have a partly cationic form at a pH value of 3.2 to 3.4. Warming up the mash also added to the higher yield of total polyphenols through enhancement of the solubility of the poor water soluble polyphenols (*13*). The yield of the antioxidant capacity was about 77% for the enzymatic treated juice compared to about 56% without the enzymatic treatment.

Table 3 shows the effect of processing on strawberry purees, which were produced without and with enzymatic maceration. In spite of higher losses (due to longer mash standing time in comparison to the nonenzymated variant), the enzymatic maceration was positive regarding the stability of the purees. The enzymatically treated puree was less viscous and smoother. It remained stable during the 11 weeks of storage, while the nonenzymatically treated puree registered a water phase separation in less than 3 weeks of storage. Passing the mash through a sieve entailed no significant changes.

Pasteurization. Pasteurization of juices and purees leads to a significant decrease of ascorbic acid by 18 to 40%. (**Tables 1** and **3**). The pasteurization of purees at 85 °C for 2 min was the processing step causing the highest losses for the most parameters. The reason for this might be the small scale processing step for the puree by heating in a water bath with air contact. The highest diminution registered the ascorbic acid with about 50%, followed by the anthocyanins with an average of 20%, and total polyphenols with about 10 to 15%. The antioxidant capacity decreased only slightly during pasteurization or increased up to 12%, depending on the method. Whereas the content of total polyphenols showed distinctive losses, the antioxidant capacity registered no major differences through heating. Therefore, it can be assumed that heating leads to the formation of new antioxidant active products, for example, products of the Maillard reaction from reducing sugars and free amino acids. Thus, Maillard reaction products formed from, for example, glucose and lysine or histidine possess an antioxidant action as shown by different studies (*33, 34*).

Also in reference to the different juices, heating led to significant losses of total polyphenols and anthocyanins. A heat treatment at 85 °C for 5 s before filling showed a diminution of 2 to 6% of total polyphenols and of 9 to 14% of anthocyanins. The pasteurization in bottles in a water bath at 85 °C for 15 min resulted in comparatively higher losses of 10% on total polyphenols and 21% on anthocyanins. This confirms the experience of the industry, that the loss of valuable ingredients with the bottle pasteurization is extremely high compared with that in flash pasteurization.

Purees and pressed juices show a different behavior during processing. If **Tables 1** and **3** are being compared, a stronger loss of ascorbic acid in the purees compared with the pressed juices is obvious. This result is surprising at first glance. A reason could be the higher activities of strawberry enzymes in the purees compared to that in the pressed juices. Especially, ascorbate oxidizing enzymes, such as polyphenol oxidase (PPO) and peroxidase (POD), have to be taken into account. Both enzymes may contribute to browning during processing and storage (*35*). Ascorbate peroxidase (APX) plays an important role in the defense against hydrogen peroxide in strawberries (*36*). APXs are located in different compartments of the fruit cells (*37*). This could explain the higher loss of ascorbic acid during puree production compared to that in pressed juice.

Storage. Storage also affects the antioxidants of strawberry juices and purees. Storage of 11 weeks at 8 °C showed a considerable loss of ascorbic acid, which decreased continuously in all products. Also, the content of anthocyanins registered a strong and steady decline of 34 to 41%. Total polyphenols and antioxidant capacity showed a diminution of 2 to 8% after 11 weeks. Aaby et al. (*38*) investigated the influence of the achenes on polyphenols, ascorbic acid, and other antioxidants in strawberry purees during processing and storage at 6 and 22 °C for 8 and 16 weeks. Purees were produced from pulp, the whole fruit, and a homogenate enriched with achenes. Ascorbic acid and anthocyanins decreased rapidly during storage, whereas polyphenols and other antioxidants were more stable. Total polyphenols and antioxidative capacity were more stable in purees with achenes.

Strawberry purees are often used in so-called smoothies. In the description of those products, it is often declared that the smoothie can substitute for the fruit. This implies a nutritional equivalence of the fruit smoothie with the original fruit. The results presented here emphasize that the fruit and the corresponding puree cannot be compared easily because of unavoidable losses of ascorbic acid and anthocyanins and, to a lower degree, of colorless polyphenols.

Conclusions. To maximize the contents of antioxidant active substances and colored anthocyanins in purees and juices, raw materials should be chosen accurately and the number of processing steps should be minimized as far as possible. However, to reach a maximal yield of polyphenols, anthocyanins, and antioxidant capacity, a short enzymatic treatment of the mash is favorable. Each processing step should be executed as efficiently as possible, and long holding times should be avoided to restrict oxidation processes.

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