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ORIGINAL PAPER

Comparative study of phenolic content and antioxidant activity of strawberry puree, clear, and cloudy juices

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Abstract The aim of this study was to determine the concentrations of phenolic compounds and their antioxidant activity in different kinds of juice: clear, cloudy, and puree which were made from three different strawberry cultivars (Elkat, Kent, and Senga Sengana). The anthocyanins, *p*-coumaric acid, ellagic acid, quercetin, keampferol derivatives, (+)-catechin, proanthocyanidins content and degree of proanthocyanidin polymerization, were determined both in the fresh and after 6 months of storage at 4 and 30 °C. Freshly produced juices contained higher amounts of phenolics, especially of anthocyanins and proanthocyanidins, than those stored for 6 months at 4 and 30 °C. The processing of the clear juice showed the higher loss of all phenolic compounds. The antioxidant capacity was the smallest for clear, and the highest for the puree juices. This was assessed by measurements made with different antioxidant activity assays: ABTS and FRAP. The puree of strawberry juice had significantly higher levels of the phenolic compounds and showed more antioxidant activity than the clear and cloudy juices, before and after storage in all strawberry cultivars.

Keywords *Fragaria x ananassa* Duch · Proanthocyanidin · Phloroglucinolysis · Antioxidant activity · Storage time

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Introduction

Strawberries (*Fragaria x ananassa* Duch.) are an important crop in certain temperate area such as, Central Europe. They are widely consumed, both fresh and in processed forms such as juices, which may further be stored. These attractive fruits are favored for their excellent taste, and can be considered a very rich source of bioactive phenolic compounds including: hydroxycinnamic acids, ellagic acid, ellagitannins, flavan-3-ols, flavonols, and anthocyanins [1]. Compared with other fruits, strawberries possess high antioxidant activity [2]. Guo et al. [3] found that strawberries had 1.3 times the antioxidant activity of oranges, twice that of red grapes, five times that of apples and bananas, and thirteen times that of honeydew. Antioxidant activity of strawberry phenolics could participate in the prevention of cancer, cardiovascular and other chronic diseases [4]. Recently, the antioxidant activity of strawberry extracts, independent of in vitro antiproliferative activity, has been shown with the use of the HepG2 human liver cancer cells [5]. Reduction in antioxidant activity during processing and storage [6] may reduce the beneficial effects of such food products on health. Injury of raw material tissue and exposure to oxygen, enzymes, light, and heat may reduce the antioxidant compound content as well. Strawberry phenolics such as pelargonidin, ellagic acid, *p*-coumaric acid, quercetin, and keampferol derivatives are very instable and undergo destruction during fruit transformation, especially during the juice and nectar production process.

Previous studies have focused on the free phenolic content and antioxidant activity of strawberry products [7, 8]. However, antioxidants can exist in both free and bound forms. Free anthocyanins, hydroxycinnamic acids, (+)-catechin, and flavonol glycoside do not bind to strawberry cell walls while proanthocyanidins polymers bound selectively

to polysaccharides and proteins before an example of this is in apples [9].

Proanthocyanidins are also found in strawberry fruits as procyanidin and propelargonidin derivatives, (+)-catechin and (−)-epicatechin contribute 93.8% of constituent units, which is in accordance with the predominance of the procyanidins in strawberry [10].

Herbert et al. [11] suggested that proanthocyanidin content in strawberries can be used as an indicator of gray mold resistance, and can be used to screen strawberry selections and cultivars in order to improve their shelf-life and quality. The proanthocyanidins are receiving increasing attention owing to their much more potent antioxidant properties than those of simple monomeric phenolics, which may correspond to a health-protective action [12]. Little is known about the structural features that affect the bioavailability and metabolism of proanthocyanidins within the body. Sano et al. [13] showed that some oligomeric proanthocyanidins are absorbed, and bioavailable in the human body, can be detected in human plasma as early as 2 h after ingestion of grape seed extract. Trimers have been shown to be absorbed through the human intestinal cell line Caco-2 [14]. For this reason, identification of heterogeneous proanthocyanidins, especially the low oligomers, are emphasized. Incubating proanthocyanidins in vitro with human colonic microflora can result in complete degradation to hydroxylated derivatives of phenylacetic, phenylpropionic and phenylvaleric acids [15] compounds that had previously been shown to arise from degradation of flavonoid monomers.

Quantitative data on proanthocyanidins found in the literature are often underestimated; the extraction from the crude materials is not quantitatively accurate because of the ability of proanthocyanidins to bind to cell wall polysaccharides [16]. These complexes involve the formation of H-bonds and hydrophobic interactions, the latter being favored by the existence of hydrophobic cavities and crevasses. Affinity is strongly influenced by the molecular weight of polyphenols, and chemical composition of polysaccharides [9].

The phloroglucinolysis reaction can be applied directly to crude strawberry materials to give pertinent information on the structure and concentration of proanthocyanidins. This method has been chosen here for the determination of procyanidins in puree, clear and cloudy strawberry juices.

The aim of this study was to determine the types and amounts of phenolic compounds in different strawberry juices and to correlate these data with antiradical activity. Our objective was to monitor changes in phenolic content, antioxidant activity during the processing and storage and storage at different temperatures of the juices obtained from three different strawberry cultivars (Elkat, Kent, and Senga Sengana which are commonly used in the Polish juice industry). The effect of clarification and solid particles content in strawberry juices on phenolic content and antioxidant activity after processing and storage was determined.

Materials and methods

Chemicals

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2′azinobis-(3-ethylbenzthiazoline-6-sulphonic acid (ABTS); potassium persulfate, (−)-epicatechin, (+)-catechin, acetic acid, benzyl mercaptan (toluene α-thiol), and methanol were purchased from Sigma-Aldrich (Steinheim, Germany). Chlorogenic acid, elagic acid, *p*-coumaric acid, quercetin, kaempferol, cyaniding-3-glucoside, pelargonidin-3-glucoside, pelargonidin-3-rutinoside were purchased from Extrasynthese (Lyon Nord, France).

Plant material

Samples of three strawberry (*Fragaria x ananassa*) cultivars (Elkat, Kent and Senga Sengana) were harvested (in Mościsko near Wrocław, Poland) at processing maturity and the fresh fruits were processed in lab scale at Wroclaw University on June 2006. Prior to juices preparation the samples were washed and carefully sorted for the same degree of maturity, size and color.

Preparation of fresh strawberry to polyphenol analysis

After harvest, the whole strawberry fruits (about 1 kg for each variety) were cut directly in liquid nitrogen, and freeze-dried (24 h). The homogeneous powder was obtained by crushing the dried tissues with the use of closed laboratory mill to avoid hydration and analyzed.

Preparation of strawberry puree, cloudy and clear juices

The whole fresh strawberry fruits (2 kg each cultivars for each replication) were ground in a Thermomix laboratory mill (Wuppertal, Vorwerk, Germany) for 20 s at exactly the same rotation speed for each samples. The 2 kg mush was mixed with 2 kg distilled water, homogenized to obtain and homogeneous pulp and divided in two parts. First part (1,200 g) was heated in a microwave oven (Amica Wronki, Poland) of 700 W for 5 min and the product temperature after treatment was of 90 °C. The hot filling was poured into 0.2 L jars with meniscus and cooled to 20 °C. This sample was called as puree juice. The second part was immediately pressed to yield 75% (weight) juice in a Zodiak laboratory hydraulic press with pressed cloth (SRSE, Warsaw, Poland). The juice was heated in a microwave oven for 5 min and the

product temperature after treatment was of 90 °C. The juice was divided in two lots. For the first lot (cloudy strawberry juice) was poured into 0.2-L jars in the same way as puree juice. The second lot (clear juice), was taken after the microwave heating, cooled to 45 °C, eventually treated with pectinase and stirred for 0.5 h at 40 °C (0.2 mL/kg Pectinex Color; Novo Nordisk Ferment Ltd, Dittingen, Switzerland). Following enzymatic treatment, the juice was clarified, using: Gelatin (0.25 g/L) + Baykisol 30 (6.0 mL/L) + SIHA-Active-Bentonite G (1.0 g/L) (Begerow GmbH&Co, Germany). This sample was then centrifuged at 12,100×*g* for 10 min and the clear juice was then pasteurized in the same way as the puree and cloudy juice. All products used for clarification were prepared by methods proposed by the producer. The amounts of each clarification product were chosen after preliminary testing. Three replicates of strawberry puree and juices preparation were carried out. After processing and storage at 4, or 30 °C in the dark, for 6 months, the strawberry products were subjected to analyses.

HPLC analysis of polyphenols

Before analysis the strawberry products were mixed with methanol (1:1) and centrifuged at 20,878×*g*. The analysis of anthocyanins, flavan-3-ols, phenolic acids and flavonol glycosides was carried out with a L-7455 liquid chromatography with a diode array detector and an L-7100 quaternary pump equipped with a D-7000 HSM multisolvent delivery system (Merck-Hitachi, Tokyo, Japan). Separation was performed in a Phenomenex (Torrance, CA, USA). Synergi Fusion RP-80A column (250 mm × 4.6 mm, 4 μm); the oven temperature was set at 30 °C. The mobile phase was composed of solvent A (45 mL formic acid and 955 mL water) and solvent B (acetonitrile). The program began with a linear gradient from 0 to 25% B in 36 min, followed by washing and reconditioning of the column. The flow rate was 1 mL/min and the runs were monitored at wavelengths of 280 nm (flavan-3-ols), 320 nm (hydroxycinnamates), 360 nm (flavonol glycosides and ellagic acid), and 510 nm (anthocyanins). Photodiode array spectra were measured over the wavelength range 200–600 nm in steps of 2 nm. Retention times and spectra were compared with those of pure standards within that range.

The amounts of the different phenolics in the samples were determined by HPLC. Calibration curves were constructed with (+)-catechin, *p*-coumaric acid, ellagic acid, isoquercitin and pelargonidin-3-*O*-glucoside as standards (Extrasynthese, France).

Analysis of proanthocyanidins by phloroglucinolysis

Direct phloroglucinolysis of freeze-dried strawberry juices was performed as described by Kennedy et al. [17]. Portions (0.5 mL) of juices were precisely measured into 2.2 mL Eppendorf vials and freeze-dried, then 0.8 mL of the methanolic solution of phloroglucinol (75 g/L) and ascorbic acid (15 g/L) were added. After addition of 0.4 mL of methanolic HCl (0.3 M), the vials were closed and incubated for 30 min at 50 °C with vortexing every 10 min. The reaction was stopped by placing the vials in an ice bath, with drawing of 0.5 mL of the reaction medium and diluting with 0.5 mL of sodium acetate buffer 0.2 M. Next the vials were cooled in ice water and centrifuged immediately at 20,000×*g* for 10 min at 4 °C. Samples were stored at 4 °C before reverse phase HPLC (RP-HPLC) analysis. All incubations were done in triplicate. Phloroglucinolysis products were separated in a Atlantis T3, 5-μm, 100A column (250 mm × 4.6 mm; Waters). The liquid chromatography was a Waters (Milford, MA, USA) system equipped with diode array and scanning fluorescence detectors. Solvent A (25 mL aqueous acetic acid and 975 mL water) and solvent B (acetonitrile) were used in the following gradient: initial, 5% B; 0–15 min, 10% B linear; 15–25 min, 60% B linear; followed by washing and reconditioning of the column. A flow rate of 1 mL/min and an oven temperature of 15 °C were observed with the injection of the filtrate (20 μL) on the HPLC system.

Compounds, for which reference standards were available, were identified on chromatograms according to their retention times and UV–visible spectra. The fluorescence detection was recorded at excitation wavelength 278 nm and emission wavelength 360 nm. The calibration curves which were based on peak area were established using (+)-catechin, (−)-epicatechin, (+)-catechin and (−)-epicatechin–phloroglucinol adducts standards. The average degree of polymerization was measured by calculating the molar ratio of all the flavan-3-ol units (phloroglucinol adducts + terminal units) to (−)-epicatechin and (+)-catechin which correspond to terminal units. Quantification (mg/L of nectars) of the (+)-catechin, (−)-epicatechin, (+) catechin and (−)-epicatechin–phloroglucinol adducts was achieved by using the calibration curves of the corresponding catechin and procyanidin standards (Extrasynthese France) (Fig. 1).

Extraction of polyphenol compounds for antioxidant activity analysis

About 10 g of each puree, clear and cloudy juices were weighed into a test tube for antioxidant property analysis. A total of 25 mL of 80% aqueous solution of methanol with 1% HCl was added, and the suspension was stirred slightly. Tubes were sonicated twice for 15 min and left at 4 °C. After 24 h the extract was centrifuged for 10 min (10 min, 1,500×*g*), and supernatants were collected at 4 °C until use (within 24 h).

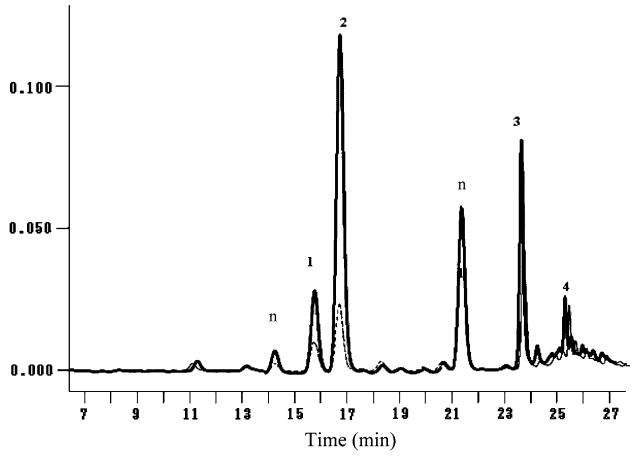

Fig. 1 Comparison of chromatograms (HPLC-FD) strawberry puree and clear juice (*dotted line*) after phloroglucinolysis for fresh Elkat cultivar. *1* Phloroglucinol–(+)-catechin; *2* phloroglucinol–(−)-epicatechin; *3* (+)catechin; *4* (−)epicatechin; *n* nonidentified

$ABTS^{\cdot+}$ radical scavenging spectrophotometric assay

The free-radical scavenging activity was determined by ABTS radical cation decolorization assay according to the method of Re et al. [18]. The ABTS was dissolved in water to a 7 mM concentration. The ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration). Next and the mixture was left to stand in the dark at room temperature (20 ± 2 °C) for 12–16 h before the use. The radical was stable in this form for more than 2 days when stored in dark at room temperature. Before analysis the $ABTS^{\cdot+}$ solution was diluted with bidistilled water to an absorbance of 0.700 (±0.02) at 734 nm. Aliquots of 30 μL of sample extract were added supernatant to 3.0 mL of diluted $ABTS^{\cdot+}$ solution [A734 nm = 0.700 (±0.02)] and the absorbance was read exactly 6 min after initial mixing. All determinations were performed in triplicate. Standard curve was prepared using different concentrations of Trolox. The results of the assay were expressed as Trolox equivalent antioxidant capacity (TEAC).

Ferric reducing/antioxidant power assay

The total antioxidant power of extracts was determined using the ferric reducing ability of plasma (FRAP) assay by Benzie et al. [19]. Briefly, the FRAP reagent was prepared by mixing acetate buffer (300 μM, pH 3.6), a solution of 10 μM TPTZ in 40 μM HCl, and 20 μM $FeCl_3$ at 10:1:1 (v/v/v). The FRAP reagent (300 μL) and sample extracts (10 μL) were added to each well and mixed thoroughly. The absorbance was taken at 593 nm after 10 min. Standard curve was prepared using different concentrations of Trolox. All solutions were used on the day of preparation. All determinations were performed in triplicates. The results were corrected for dilution and expressed in μM Trolox/100 g dry weight (dw).

Statistical analyses for individual phenolics in strawberry products

Statistical analysis was conducted using Statistica version 6.0 (StatSoft Poland). Significant differences ($P \leq 0.05$) between average responses were evaluated by using one-way ANOVA with Duncan test. Principal component analysis (PCA) was performed using XLSTAT (Addinisoft, France) was performed on mean values of 27 samples and 5 variables.

Results and discussion

The puree, clear and cloudy juice of strawberry were obtained from three cultivars, Elkat, Kent, and Senga Sengana. The results of the qualitative and quantitative phenolic composition of the strawberry juices, as determined by the HPLC method, are presented in Tables 1, 2, and 3. The concentrations of anthocyanins, *p*-coumaric acid, ellagic acid, quercetin, keampferol derivatives, (+)-catechin, and proanthocyanidins as well as degree of polymerization of proanthocyanidins were determined in raw material, fresh products and after 6 months of storage at 4 and 30 °C.

The average phenolic contents of the three strawberry cultivars, Elkat, Kent and Senga Sengana used as raw material are shown in Table 1. The total phenolic content (considering the sum of all of the individual phenolics) ranged from 243.3 mg/kg fresh fruits (Senga Sengana) to 290.4 mg/kg fresh fruits (Elkat, typically Polish cultivar). Proanthocyanidins were the major polyphenols in all the samples under study. Regarding proanthocyanidins, the highest concentrations were found in Kent (235.5 mg/kg fresh fruits) and the smallest in Senga Sengana (159.2 mg/kg fresh fruits) cultivars. Ellagic acid is a hydrolytic product of ellagitannins. The content of total ellagic acid (ellagic acid and ellagic acid glucoside) ranged from 1.2–1.3 mg/kg fresh fruits (Senga Sengana and Kent) to 3.5 mg/kg fresh fruits (Elkat). The concentration of ellagic acid and ellagic acid glucoside was the present at same as previously reported in strawberry fruits [1, 7]. The concentration of flavonols such as kaempferol and quercetin ranged from 5.1 to 9.0 mg/kg. Lugasi and Hovari [20] reported that quercetin was present at a concentration of 10.0–53.0 mg/kg. Variation in content of different phenolic in strawberries may be due to cultivar between maturity, size, present of achenes, extraction solvent and procedure. Pelargonidin-3-*O*-glucoside was predominant in all cultivars and ranged from 29.9 mg/kg

Table 1 Concentration (mg/kg of fresh weight) of the phenolic compounds in raw material used for strawberry products: clear, cloudy and purees juices

Phenolic compound	Senga Sengana	Kent	Elkat
Cyanidin-3-*O*-glucoside	2.9 ± 0.3f	2.0 ± 0.4e	3.4 ± 0.7f
Pelargonidin-3-*O*-glucoside	53.9 ± 3.2b	29.9 ± 1.2b	68.2 ± 3.8b
Pelargonidin-3-*O*-rutinoside	3.9 ± 0.5e	1.9 ± 0.7e	0.0 ± 0.0 g
Pelargonidin-3-*O*-malonyl-glucoside	6.9 ± 1.3c	4.7 ± 0.3d	12.4 ± 0.3c
p-Coumaric acid	6.9 ± 0.9c	1.3 ± 0.4f	8.1 ± 1.9d
Ellagic acid	1.2 ± 0.3 g	1.3 ± 0.6f	3.5 ± 0.8f
Flavonols[a]	3.5 ± 0.8e	9.0 ± 1.8c	5.1 ± 0.5e
(+)-Catechin	4.9 ± 1.0d	5.0 ± 0.4d	5.8 ± 1.1e
Proanthocyanidins	159.2 ± 4.7a	235.5 ± 5.8a	183.9 ± 3.8a
Total	243.3	290.6	290.4

[a] Flavonols (sum of kaempferol and quercetin)

Table 2 Mean contents of phenolic compounds (in mg/L ± SD) in clear, cloudy and puree strawberry juices during 6 months of storage in different conditions (4 and 30 °C)

Cultivars	Time and conditions of storage	*p*-Coumaric acid	Ellagic acid	Quercetin	Kaempferol	(+)-Catechin	Proanthocyanidins	Degree of polymerization of proanthocyanidins
Senga Sengana	C-0M	29.4 ± 0.2d[a]	3.3 ± 0.4i	1.9 ± 0.3jk	1.8 ± 0.5b	20.3 ± 0.4efg	157.3 ± 2.3s	2.60r
	T-0M	34.9 ± 0.4ab	2.4 ± 0.0ijk	1.5 ± 0.0jk	6.3 ± 0.2a	23.4 ± 1.6d	188.4 ± 7.4n	3.67m
	P-0M	35.9 ± 1.1a	15.8 ± 1.3ef	3.1 ± 0.7ij	6.7 ± 0.5a	25.6 ± 2.4c	452.7 ± 3.4c	4.12k
	C-6M-L	30.1 ± 1.1d	2.2 ± 0.3ijk	0.7 ± 0.1k	1.3 ± 0.1b	18.7 ± 1.8gh	93.9 ± 4.3x	4.97i
	T-6M-L	34.1 ± 1.0b	2.6 ± 0.2ijk	1.4 ± 0.2jk	1.3 ± 0.3b	21.6 ± 1.2e	172.8 ± 6.5o	7.43b
	P-6M-L	32.4 ± 2.3c	28.9 ± 1.9b	1.4 ± 0.3jk	2.3 ± 0.6b	20.5 ± 2.3ef	412.6 ± 1.8f	9.22a
	C-6M-H	19.4 ± 3.2h	1.0 ± 0.1k	0.5 ± 0.0k	1.3 ± 0.7b	7.9 ± 1.2op	69.1 ± 1.1z	2.34s
	T-6M-H	22.0 ± 0.1g	1.3 ± 0.0jk	0.7 ± 0.0k	0.9 ± 0.1b	17.3 ± 2.3hi	136.1 ± 1.9w	5.61g
	P-6M-H	19.5 ± 0.0h	22.5 ± 1.4c	1.0 ± 0.0k	2.7 ± 0.8b	18.8 ± 4.5fgh	276.2 ± 0.5r	6.85d
Elkat	C-0M	24.2 ± 0.2ef	6.9 ± 0.5g	3.0 ± 0.2ij	0.8 ± 0.0b	14.0 ± 2.3jk	264.2 ± 2.9k	2.60r
	T-0M	25.7 ± 0.9e	2.3 ± 1.0ijk	8.2 ± 1.0de	0.8 ± 0.0b	13.4 ± 1.2kl	316.5 ± 3.4i	3.67m
	P-0M	28.9 ± 1.9d	16.3 ± 2.2e	12.3 ± 1.7a	1.5 ± 0.1b	15.8 ± 0.8ij	592.5 ± 2.9a	4.12k
	C-6M-L	22.7 ± 1.2fg	6.9 ± 0.7g	1.2 ± 0.3k	1.5 ± 0.4b	13.4 ± 1.4kl	167.9 ± 4.5p	2.73pr
	T-6M-L	23.0 ± 1.3fg	2.2 ± 0.3ijk	7.1 ± 0.6ef	1.3 ± 0.2b	14.8 ± 1.6jk	224.3 ± 7.8l	3.58m
	P-6M-L	25.2 ± 2.3e	32.5 ± 2.1a	11.6 ± 2.3ab	1.8 ± 0.6b	15.8 ± 2.3ij	498.6 ± 0.9b	5.15h
	C-6M-H	16.3 ± 1.9j	3.9 ± 0.5hi	0.9 ± 1.1k	1.7 ± 0.5b	4.1 ± 0.5r	136.5 ± 2.3w	2.07f
	T-6M-H	18.0 ± 2.9hi	2.0 ± 0.3ijk	2.2 ± 0.2jk	1.8 ± 0.3b	6.6 ± 0.7p	167.1 ± 1.2p	3.03o
	P-6M-H	7.7 ± 0.0ij	23.3 ± 0.2c	0.8 ± 0.0k	2.2 ± 0.5b	7.7 ± 0.9p	404.3 ± 1.9g	4.33j
KENT	C-0M	2.9 ± 1.8l	5.9 ± 1.1g	1.1 ± 0.3k	1.0 ± 0.6b	28.4 ± 1.5b	172.4 ± 9.2o	3.24n
	T-0M	3.0 ± 1.1kl	3.4 ± 1.8i	6.4 ± 0.2fg	1.5 ± 0.2b	26.0 ± 1.2c	197.7 ± 6.7n	3.94l
	P-0M	3.8 ± 0.4k	14.5 ± 1.4f	10.1 ± 0.1bc	2.2 ± 0.0b	39.4 ± 2.3a	449.7 ± 10.1d	7.12c
	C-6M-L	2.3 ± 1.0kl	5.6 ± 0.5gh	1.1 ± 0.0k	1.0 ± 0.2b	9.5 ± 2.8no	160.7 ± 1.7r	2.82p
	T-6M-L	3.1 ± 1.9kl	2.9 ± 0.1ij	5.6 ± 1.1fgh	1.4 ± 0.9b	11.8 ± 1.8lm	182.7 ± 7.9n	3.70m
	P-6M-L	3.2 ± 1.8kl	28.7 ± 5.6b	9.4 ± 1.6cd	2.0 ± 0.6b	20.0 ± 2.8efg	433.4 ± 1.8e	6.52e
	C-6M-H	1.8 ± 1.0l	3.1 ± 2.1ij	0.5 ± 0.1k	1.1 ± 0.2b	6.7 ± 2.9op	80.9 ± 1.5g	1.95t
	T-6M-H	2.4 ± 1.0kl	5.2 ± 2.3gh	4.0 ± 0.2hi	1.2 ± 0.0b	10.2 ± 1.9mn	147.7 ± 0.3t	3.33n
	P-6M-H	2.2 ± 0.7kl	20.2 ± 0.1d	5.2 ± 0.6gh	2.0 ± 0.7b	8.0 ± 0.1op	367.0 ± 1.1 h	5.93f

C Clear juice, *T* cloudy juice, *P* puree; *M* months; *L* 4 °C; *H* 30 °C

[a] Values are mean ± standard deviation, $n = 3$; in each column, mean values with different letters are significantly different at $P < 0.05$

for Kent strawberries to 68.2 mg/kg for Elkat. Significant differences in the concentration of *p*-coumaric acid among different cultivars (1.3 mg/kg fresh fruits for Kent and 8.1 mg/kg fresh fruits for Elkat) were found. The concentration of *p*-coumaric acid found here in Senga Sengana was twice that reported by Häkkinen and Törrönen [21].

Table 3 Mean contents of anthocyanins (in mg/L ± SD) in clear, cloudy and puree strawberry juices during 6 months of storage in different conditions (4 °C and 30 °C)

Cultivars	Time and conditions of storage	Cyanidin-3-glucoside	Pelargonidin-3-glucoside	Pelargonidin-3-rutinoside	Pelargonidin-3-malonylglucoside	Total anthocyanins
Senga Sengana	C-0M	9.5 ± 0.3bc[a]	218.7 ± 4.3d	9.7 ± 0.0a	14.9 ± 0.2g	252.8c
	T-0M	9.7 ± 0.0bc	228.0 ± 1.9c	10.3 ± 1.4a	24.9 ± 1.7d	272.9c
	P-0M	11.8 ± 1.8a	248.6 ± 1.8a	10.6 ± 2.9a	27.7 ± 1.7c	298.7a
	C-6M-L	0.5 ± 0.1r	9.5 ± 1.9x	1.2 ± 0.1g	0.3 ± 0.1l	133.3k
	T-6M-L	0.5 ± 0.0r	22.2 ± 4.3s	1.5 ± 1.0fg	0.5 ± 0.2kl	165.2h
	P-6M-L	1.1 ± 0.1ij	29.7 ± 1.1r	1.4 ± 0.0g	0.7 ± 0.0kl	184.5g
	C-6M-H	4.2 ± 0.1fg	118.7 ± 1.9k	5.5 ± 1.8c	5.0 ± 0.1j	11.5t
	T-6M-H	6.7 ± 0.6d	141.5 ± 1.6h	6.8 ± 0.3b	10.1 ± 1.1h	24.6s
	P-6M-H	8.9 ± 0.5bc	154.5 ± 2.9g	7.0 ± 0.4b	14.2 ± 0.3g	32.9r
Elkat	C-0M	8.8 ± 1.1bc	207.6 ± 3.2f	0.0 ± 0.0h	22.4 ± 0.4e	238.7f
	T-0M	8.8 ± 1.8bc	216.3 ± 2.3e	0.0 ± 0.0h	33.5 ± 0.6b	258.6d
	P-0M	0.4 ± 0.4ab	237.2 ± 5.6b	0.0 ± 0.0h	37.4 ± 1.1a	285.0b
	C-6M-L	4.1 ± 0.4fg	117.8 ± 1.7k	0.0 ± 0.0h	10.5 ± 1.0h	132.4k
	T-6M-L	5.8 ± 0.3def	129.1 ± 1.9j	0.0 ± 0.0h	13.5 ± 1.8g	148.4j
	P-6M-L	8.3 ± 0.2c	135.7 ± 1.4i	0.0 ± 0.0h	17.4 ± 1.5f	161.4i
	C-6M-H	1.0 ± 0.1ij	6.0 ± 0.3y	0.0 ± 0.0h	2.5 ± 0.4k	9.4y
	T-6M-H	0.7 ± 0.1ij	11.5 ± 0.1w	0.0 ± 0.0h	2.3 ± 0.4kl	14.6w
	P-6M-H	1.1 ± 0.1ij	14.1 ± 1.6t	0.0 ± 0.0h	1.1 ± 0.1kl	16.3t
Kent	C-0M	4.9 ± 0.4efg	102.1 ± 1.5c	3.8 ± 2.7de	7.4 ± 0.3i	118.2m
	T-0M	4.6 ± 0.2efg	99.2 ± 3.8m	3.9 ± 0.2de	14.2 ± 2.0g	121.9l
	P-0M	6.0 ± 1.1de	119.1 ± 8.1k	4.3 ± 0.7cd	17.7 ± 2.7f	147.1j
	C-6M-L	3.9 ± 0.5gh	56.4 ± 3.6p	2.9 ± 0.3de	4.1 ± 1.1j	67.2p
	T-6M-L	2.4 ± 0.7hi	69.9 ± 2.9o	2.7 ± 0.7ef	6.7 ± 1.7i	81.7o
	P-6M-L	4.8 ± 0.4efg	83.2 ± 5.2n	2.9 ± 0.2de	11.4 ± 0.3h	102.3n
	C-6M-H	0.0 ± 0.0j	1.1 ± 0.5z	0.1 ± 0.0h	0.2 ± 0.0l	1.5z
	T-6M-H	0.1 ± 0.0j	6.1 ± 0.6y	0.1 ± 0.0h	1.5 ± 0.3kl	7.8y
	P-6M-H	0.2 ± 0.1j	7.2 ± 0.2y	0.3 ± 0.0h	1.1 ± 0.1kl	8.8y

$n = 3$; in each column, mean values with different letters are significantly different at $P < 0.05$

C Clear juice, *T* cloudy juice, *P* puree; *M* months; *L* 4 °C; *H* 30 °C

[a] Values are mean ± standard deviation

Out of the three kinds of strawberry juices, the phenolic compounds that showed the biggest difference were proanthocyanidins (Tables 2, 3). The amount of these compounds differed significantly between clear and puree juices (i.e. cloudy juice still containing a suspension of cell-wall fragments and cumulative), with a two- to three-fold variation of their concentration. The fresh clear juice from Senga Sengana, Elkat, and Kent cultivars contained 157.3, 264.2, and 172.4 mg/L of proanthocyanidins, respectively. While the puree juice from Senga Sengana, Elkat, and Kent cultivars contained 452.7, 592.5, and 449.7 mg/L, respectively. The degree of polymerization (DP) was higher for puree (DP 4.1–7.1) than for clear juice (DP 2.6–3.2). Strawberry polymeric flavan-3-ols are composed of (+)-catechin and (−)-epicatechin which are constitutive units of procyanidins. The chain extension units and chain terminating units in procyanidins were (+)-catechin and (−)-epicatechin (Fig. 2). Polymeric proanthocyanidins are the major class of polyphenolic compounds in strawberry puree juice. The concentration of proanthocyanidins was present at much higher levels as previously reported for strawberry [22]. This difference is due to the fact that our results take into account the polymer and oligomer proanthocyanidins analysed by phloroglucinolysis method. Previous studies had reported only oligomer proanthocyanidin content analysed directly by HPLC method [23, 24]. Puree juice also contained the highest amount of free ellagic acid. The average concentration ranged from 14.5 mg/L in Kent puree juice up to 16.3 mg/L in Elkat puree juice. After 6 months of storage

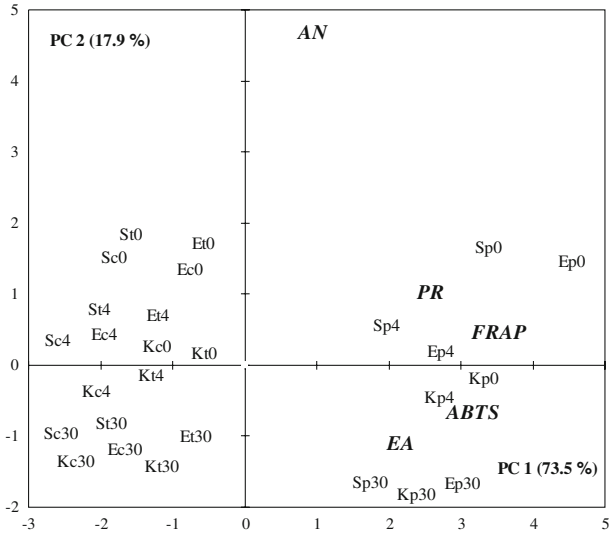

Fig. 2 Principal component analysis of strawberry juice (*c* clear; *t* cloudy; *p* puree) prepared from three cultivars (*S* Senga Sengana; *E* Elkat; *K* Kent) before storage time (0) and after stored 6 months at 4 (4) and 30 °C (30). The measured variables (phenolics: *AN* anthocyanins; *PR* proanthocyanins; *EA*ellagic acid; antioxidant capacity: *ABTS* and *FRAP*) are shown on the same plot

at 4 °C, ellagic acid in Kent and Elkat puree juices increased significantly to 2.87 and 32.5 mg/L, respectively.

Proanthocyanidins and ellagitannins are present at higher concentrations in the puree juice (Tables 1, 2) than in fresh fruits. Ellagic acid occurs in particularly high concentration in strawberry achenes [23] and it is liberated as a hydrolytic product of ellagitannins in puree juices during processing and storage.

The processing of puree, and clear and cloudy juices, had a less effect on (+)-catechin, anthocyanin, *p*-coumaric acid, quercetin, and keampferol derivative content (Table 2, 3). The differences due to heterogeneity of raw material were limited by preparing the strawberry products from the same homogenate strawberry pulps. Qualitative differences were found in anthocyanin composition of the three cultivars. Four anthocyanins were identified in Senga Sengana and Kent strawberry cultivars: cyanidin-3-*O*-glucoside, pelargonidin-3-*O*-glucoside, pelargonidin-3-*O*-rutinoside, and pelargonidin-3-*O*-malonylglucoside. Elkat cultivar (Polish origin) had only three anthocyanins which did not include the pelargonidin-3-*O*-rutinoside. The content of anthocyanidins in strawberries and, then, in juices depended on a series of factors, such as the stage of maturity, cultivar, storage conditions. Pelargonidin-3-*O*-glucoside was predominant in all strawberry products and ranged from 248.6 mg/L for Senga Sengana purée juice to 99.2 mg/L for Kent cloudy juice. The process had less influence on the anthocyanidins concentrations than the storage did (Tables 1, 3). All samples exhibited a dramatic

Table 4 Antioxidant activities of strawberry juices as determined by the ABTS and FRAP assays (μM Trolox/100 mL) before and after 6 months of storage in different conditions (4 and 30 °C)

Variety	Time and conditions of storage	ABTS	FRAP
Senga Sengana	C-0M	80.83 ± 1.34k	335.39 ± 1.43r
	T-0M	73.06 ± 3.56m	380.11 ± 2.13n
	P-0M	200.65 ± 2.00c	1021.26 ± 1.24b
	C-6M-L	73.58 ± 1.43m	302.77 ± 3.56s
	T-6M-L	69.43 ± 1.45n	364.87 ± 2.87o
	P-6M-L	187.86 ± 1.11e	893.55 ± 2.87e
	C-6M-H	53.89 ± 2.67r	261.72 ± 2.56x
	T-6M-H	55.96 ± 1.65s	306.63 ± 3.01s
	P-6M-H	107.36 ± 2.03g	630.06 ± 1.18g
Elkat	C-0M	88.60 ± 2.87j	470.82 ± 2.45i
	T-0M	101.04 ± 1.23h	514.67 ± 1.34
	P-0M	248.54 ± 2.90a	1155.86 ± 4.67a
	C-6M-L	78.76 ± 1.11l	295.92 ± 2.01t
	T-6M-L	96.89 ± 3.02i	410.30 ± 2.56l
	P-6M-L	195.83 ± 1.34d	712.84 ± 3.05f
	C-6M-H	59.84 ± 1.09p	415.56 ± 1.17k
	T-6M-H	69.69 ± 2.78n	510.69 ± 1.45h
	P-6M-H	149.54 ± 2.45f	928.75 ± 1.46d
Kent	C-0M	69.43 ± 1.45n	390.13 ± 1.92m
	T-0M	95.85 ± 1.23i	511.16 ± 1.83h
	P-0M	205.20 ± 2.98b	957.38 ± 1.89b
	C-6M-L	68.91 ± 1.11n	356.81 ± 0.21o
	T-6M-L	79.79 ± 1.67l	465.74 ± 3.00j
	P-6M-L	193.91 ± 1.12d	924.06 ± 2.56d
	C-6M-H	51.81 ± 0.23t	271.80 ± 1.35w
	T-6M-H	63.21 ± 1.01o	369.08 ± 1.78o
	P-6M-H	145.36 ± 1.59f	713.31 ± 1.09f

$n = 3$; in each column, mean values with different letters are significantly different at $P < 0.05$

C Clear juice, *T* cloudy juice, *P* puree juice; *M* months; *L* 4 °C; *H* 30 °C

[a] Values are mean ± standard deviation

anthocyanidins loss when stored at the highest temperature (30 °C) and much slower at 4 °C (Table 3). All strawberry products stored for 6 months at 30 and at 4 °C had about one-tenth and half of the initial concentrations, respectively. The results were consistent with general findings that monomeric pigment concentrations decrease during storage [24, 25] and the stability of anthocyanins was markedly influenced by temperature [26, 27].

Some protective effects on anthocyanidins degradation were observed in puree and cloudy juice in comparison to clear juice. The presence of pectins in cloudy has protected strawberry juices against anthocyanin degradations. Other factors could be connected with co-pigmentation by high concentrations of copigments, such as proanthocyanidins,

Table 5 Correlation coefficients of anthocyanins, procyanidins, ellagic acid and total antioxidant activity measured by ABTS and FRAP

Variables	Anthocyanins	Proanthocyanidins	Ellagic acid	ABTS	FRAP
Anthocyanins		**0.428**	0.055	0.172	0.306
Proanthocyanidins	**0.428**		**0.805**	**0.870**	**0.944**
Ellagic acid	0.055	**0.805**		**0.729**	**0.771**
ABTS	0.172	**0.870**	**0.729**		**0.910**
FRAP	0.306	**0.944**	**0.771**	**0.910**	

Values in *bold* are significantly different from 0 with a significance level alpha = 0.05

which can be involved π–π stacking (co-pigmentation) and formation of brownish covalent adducts [28, 29].

The concentrations of phenolics such as *p*-coumaric acid, quercetin, keampferol derivatives, and (+)-catechin were lower than those of anthocyanins and proanthocyanidins in both fresh fruits and juices from all three strawberry cultivars (Tables 1, 2). The puree juice was richer in these phenolics than the clear and cloudy ones. Juices prepared from Elkat and Senga Sengana contained about ten times more *p*-coumaric acid than those prepared from Kent strawberry, especially for puree juices.

The concentration of proanthocyanidins and other phenolic compound in the cloudy and puree juice was higher than clear juice independently of the variety. The transfer rates varied between the type of processing and also between the cultivars. The increased transfer of proanthocyanidins from clear to cloudy juice then to puree was accompanied by higher degree of polymerization, and this is in agreement with data for apple obtained by Le Bourvellec et al. [30].

All juices were examined for their antioxidant activity. The antioxidant activities of clear and cloudy juices and puree juice, assessed either by a radical scavenging assays (ABTS) or a reducing power assay (FRAP) were significantly different (Table 4). The process strongly affected the antioxidant activity and this was closely dependent on the content of phenolic compounds, as was confirmed by Fernandez-Pachon et al. [31] in their study on wine-process. Significant differences in the antioxidant activity among clear, cloudy, and puree juices were also found after 6 months of storage at 4 and 30 °C. Clear, cloudy and puree produced from all cultivars stored at 4 °C had significantly higher antioxidant capacity than products stored at 30 °C. Our results are consistent with the results obtained by Wicklund et al. [32] for strawberry jams stored for 3 months at 4 and 20 °C. Moreover, differences were ascertained between varieties Senga Sengana juices had the lowest ABTS and FRAP-values. In Wicklund et al. [32] strawberry jam preserves prepared in laboratory with the Senga Sengana variety had smaller FRAP.

The need for the use of different methods/assay to measure the antioxidant activity is due to the different chemical mechanisms beyond each assays and to the fact that antioxidants could acts through different modes of action [33]. The use of a single method provides only an estimate of the capacity that is dependent upon time of reaction, mode of action, and the complexity of the reaction kinetics. Second, the potential for interaction/polymerization of phenolic compounds may cause the antioxidant capacity of both fruit samples and individual compounds to be underestimated. Using at least two different antioxidant methods to compare fruits samples provides the opportunity to identify variations in response that may otherwise be missed.

Principal component analysis was preformed for all 27 samples using the mean values of six variables: three concentrations (total anthocyanins and proanthocyanidins, and ellagic acid) and the two antioxidant capacities (ABTS, FRAP). One the first PC, which explained 73.5% of the variance loaded the proanthocyanidins content, the ellagic acid content and the antioxidant activity. Along the firs PC samples of "puree juice" were separated from samples of clear and cloudy juices regardless of the variety and storage conditions. Moreover, within each single variety, cloudy juices were separated from puree juices along PC1 for a lower polyphenols content and antioxidant activity. The puree juices indeed contained higher polyphenol concentrations and had higher antioxidant capacities. On the second PC which explained 17.9% of total variation, loaded the anthocyanins content. Juices stored at different temperatures for different durations, were separated the juices stored at 30 °C. PCA permitted to evidence a very limited impact of the cultivars on data structure, with some separation of Senga Sengana, from Elkat along PC1Kent. There were significant correlations (Table 5) between proanthocyanidins concentrations and ellagic acid concentrations and the two antiradical assays, with Pearsons coefficients of 0.870 and 0.944 for ABTS and FRAP, respectively, as well as between ellagic acid and antioxidant capacity 0.729 and 0.771, respectively. However, the concentrations of proanthocyanidins and ellagic acids were also strongly correlated in this data set, in contrast to anthocyanins (Table 5). The relationship between the various phenol classes could be related to their response to the process (better transfer to the puree juice for proanthocyanidins and ellagic acid due either to the adsorption on cell wall remnants or extraction

from fragmented seeds over time; higher sensitivity to storage for anthocyanins).

Our results showed that the puree of strawberry juice had significantly higher levels of the phenolic compounds and showed more antioxidant activity than the clear and cloudy juices, before and after storage in all strawberry cultivars. Therefore puree strawberry juice may be interesting from a nutritional and, thus, commercial and pharmaceutical, perspective.

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