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Distribution of Phenolic Components and Their Antioxidant Capacity in Strawberries

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Abstract

Total phenolic content, phenolic composition, and total antioxidant capacity of neck and bottom parts of two strawberry cultivars, ‘Chambly’ and ‘LL0220-10’, were evaluated using the Folin-Ciocalteu method, high performance liquid chromatography, and ferric reducing/antioxidant power, respectively. A significant interaction ($p < 0.05$) was observed between examined parts and cultivars. The highest content of phenolics was detected in the bottom part of ‘Chambly’ when compared to its neck, while there was no difference between neck and bottom of ‘LL0220-10’. The most evident group was the anthocyanins accounting for 83.53% of the total phenolic content. These data are a step in establishing a correlation between fruit anatomy and its chemical composition, which could be useful in breeding new strawberry cultivars more resistant to diseases.

Keywords: strawberry, fruit parts, *Botrytis cinerea*, phenolic content, phenolic composition, antioxidant activity, disease resistance

1. Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is widely cultivated and very important crop in Canada. It is very delicate and perishable which makes it very susceptible to grey mold by *Botrytis cinerea*, reducing much its postharvest life. It is really difficult to control grey mold because that pathogen can infect all plant parts, mostly during the ripening process and at harvest, when the use of fungicide is not possible, which results in a shorter shelf life (Martínez-Romero et al., 2007). Thus, grey mold is the number one destructive disease for strawberry, all around the world (Sutton, 1998). There are many cultural practices which can prevent its growth, including fungicide applications, temperature and atmosphere control, better irrigation and wetness, at different growth stages and postharvest, but the best method still is the selection of cultivars with a greater resistance to its damaging effects (Hébert et al., 2002; Terry, Joyce, Adikaram, & Khambay, 2004). In view of results obtained through many experiments, researchers have attributed variations in the inherent natural disease resistance of strawberry to its skin strength (Gooding, 1976), firmness (Barritt, 1980), flower susceptibility (Bristow, Campbell, Papendick, & Elliot, 1986), raised neck, and reflexed calyx. Firm flesh strawberry seems to be more resistant to grey mold (Olcott-Reid & Moore, 1995) which is also associated to specific phenolics and total antioxidant capacity (TAC). Cultivars having different phenolic contents also have different shelf life and susceptibilities to the pathogen infection. For instance, the June-bearing genotype ‘La Clé des Champs’ has a higher total phenolic content (TPC) and lower susceptibility to postharvest disease compared with ‘FIN0132-11’, a day-neutral genotype (Khanizadeh, Ehsani-Moghaddam, & Levasseur, 2006). Phenolic compounds such as catechin, which is the main component of proanthocyanidins, are oligomers of flavan-3-ols found in unriped, green-coloured strawberries, and are related to *B. cinerea* resistance (Jersch, Scherer, Huth, & Schlösser, 1989; Feucht, Treutter, & Christ, 1992; Di Venere et al., 1998).

The main objectives of our work were to establish whether phenolic composition between fruit parts differed, and if so, to investigate its effect on disease susceptibility by grey mold.

2. Materials and Method

2.1 Chemicals

Ellagic, gallic, and *p*-coumaric acids, sodium carbonate (Na_2CO_3), and Folin-Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA), quercetin-3-galactoside from Fluka Chemie GmbH (Buchs, Switzerland), cyanidin-3-galactoside from Indofine Chemical Co. (Hillsborough, New Jersey, USA), and all other solvents from Caledon Laboratories Ltd. (Georgetown, Ontario, Canada).

2.2 Sample Preparation and Extraction Procedures

Strawberry cultivars, 'Chambly' and 'LL0220-10', which have raised neck, elevated calyces and intense red fruits with uniform and deep coloured flesh, were used in a complete randomized design using four replicates. According to previous evaluations, 'LL0220-10' is more resistant to *B. cinerea* than 'Chambly'. During the 2006-2007 harvesting seasons, fruits were picked randomly at commercial maturity, at the Agriculture and Agri-Food Canada experimental farm located in L'Acadie, Quebec, Canada (longitude 73°35′ W, latitude 45°32′ N). The harvested fruits were separated into neck and bottom parts (Figure 1), and 3-150 g lots from each part were weighed. Whole fruits samples were also used for comparison.

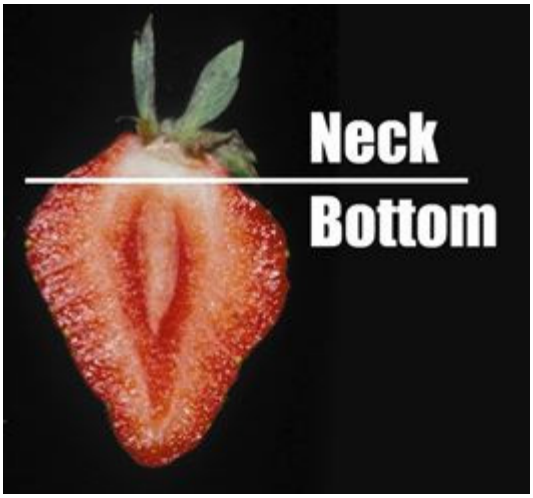

Figure 1. Strawberry parts used for analysis

All samples were cut into small pieces and frozen immediately into liquid nitrogen before being stored at -80 °C for future analysis.

From each fruit lot, 10 samples were pooled and ground in a mortar containing liquid nitrogen. These frozen samples were then mixed with 50 mL of 50% methanol with a Polytron blender (Brinkmann Instruments, New York, USA). The mixture obtained was passed through Whatman No. 1 filter paper and thereafter through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, Michigan, USA). The final filtrate was kept at -20 °C until analysis.

2.3 Total Phenolic Content, Phenolic Composition and Total Antioxidant Capacity

TPC was determined by the Folin-Ciocalteu method (Slinkard & Singleton, 1977), with slight modifications (Wang et al., 2012). Phenolic composition (PC) was analyzed by HPLC (Wang et al., 2012) and TAC was estimated by the use of the ferric reducing/antioxidant power (FRAP) (Benzie & Strain, 1996) with modifications for the 96-well microplate reader.

2.4 Sensory and Physical Evaluations

For each cultivar, five fruits were placed on top of a filter paper previously placed in a petri dish, at room temperature. Fruit weight, juice loss, glossiness and postharvest diseases were evaluated daily, until fruits were no more marketable.

Fruit firmness was assessed by use of a LRX tester (Lloyd Instruments, Brampton ON. Canada). A constant 50 N weight with a load cell sensitivity of 100.4% was applied, with a probe 3.5 cm in diameter.

Five whole fruits or fruit parts were taken, at random, to determine the soluble solids content (SSC) and titratable acidity (TA). Juice extracts were obtained by use of a Supreme Juicerator juicer (ACME Juicer Mfg. Co., New Hartford, Connecticut, USA). SSC was determined at room temperature by means of a refractometer (ABBE MARK II; Reichert-Jung, Markham, Ont., Canada). TA was determined by mixing 9 mL distilled water with 1 mL of the strawberry juice. The mixture obtained was then titrated by means of a pH meter (Accumet AB15 Basic pH meter; Fisher Scientific, Ont., Canada), using 0.1 N NaOH until pH 8.1.

2.5 Statistical Analysis

Data were analyzed by use of the ANOVA and GLM procedures of SAS (SAS Institute, 1989), where means were separated by the least significant difference (LSD), at the 0.05 level.

3. Results

Results obtained for TPC and TAC are presented in Table 1.

Table 1. Distribution of total phenolic content and total antioxidant capacity of the different fruit parts of the two selected strawberry lines

Cultivar & parts	Total phenolic content[a] (μg GAE g^{-1})	Total antioxidant capacity[b] (μg AAE g^{-1})	Weight (g)	Soluble solids content (^{o}Brix)	Titratable acidity	Firmness	pH	Shelf-life at room temperature (d)
'Chambly' Neck	774.87bc	1178.00a	17.57b	6.52bc	0.79a	-	3.90a	-
'Chambly' Bottom	1093.11a	1350.30a	51.03a	6.73abc	0.54b	-	3.60b	-
'Chambly' Whole fruit	893.34ab	1022.00a	55.55a	7.36ab	0.78a	7.5a	4.00a	2
'LL0220-10' Neck	585.09cd	1247.00a	16.34b	6.27c	0.80a	-	3.67b	-
'LL0220-10' Bottom	459.85d	1259.70a	54.57a	6.93abc	0.44bc	-	3.59b	-
'LL0220-10' Whole fruit	903.10ab	1449.30a	57.24a	7.47a	0.89a	9.6b	3.81ab	3
Mean	784.89	1265.67	42.05	6.88	0.58	10.0	3.76	-
$LSD_{0.05}$[c]	280.53	588.24	8.42	0.84	0.10	1.50	0.13	-

The Folin–Ciocalteu and ferric reducing/antioxidant power (FRAP) analyses were performed in triplicate.

[a]Total phenolic content (Folin-Ciocalteu analysis) is expressed as micrograms of gallic acid equivalent (GAE) per gram fresh-frozen weight.

[b]Antioxidant capacity (FRAP assay) is expressed as micrograms of ascorbic acid equivalent (AAE) per gram fresh-frozen weight.

[c]$LSD_{0.05}$: Least significant difference at the 0.05 level.

We obtained significant variations among the fruit parts of the two cultivars, 'Chambly' and 'LL0220-10'. TPC values varied from 459.85 - 1093.11 μg gallic acid eq. (GAE) g^{-1}. Bottom part of 'Chambly' contained higher TPC than its neck (1093.11 and 774.87 μg GAE g^{-1}, respectively). When compared to 'Chambly', both neck and bottom parts of 'LL0220-10' had lower TPC, and there were no significant differences in between them (585.09 and 459.85 μg GAE g^{-1}, respectively). Even though TPC of the samples were different, there were no significant differences between TAC values obtained (Table 1).

PC was separated into 5 components. Among those, anthocyanins were the most predominant one (83.53%),

followed by flavonols (7.89%) and hydroxycinnamic, benzoic and ellagic acids (3.66, 2.71 and 2.22%, respectively. (Table 2)

Table 2. Phenolic composition (μg g^{-1} fresh-frozen weight) of the different fruit parts of the two selected strawberry lines

Cultivar and part	Total anthocyanins (520 nm)	Total hydroxycinnamic acids (320 nm)	Total flavonols (360 nm)	Total ellagic acids (254 nm)	Total benzoic acids (280 nm)	Total phenolics (sum of five groups)[a]
'Chambly', neck	751.78ab	33.22b	85.39a	12.66ab	18.84bc	901.88b
'Chambly', bottom	869.33a	60.70a	86.32a	17.05ab	46.06a	1079.45a
'Chambly', whole fruit	695.00b	40.21b	56.50b	18.23a	23.16b	833.09b
'LL0220-10', neck	464.00c	8.09c	31.76c	17.55a	13.62bcd	535.02c
'LL0220-10', bottom	435.99c	12.80c	26.78c	12.09b	9.48cd	497.15c
'LL0220-10', whole fruit	395.44c	3.42c	53.73b	18.55a	6.13d	477.27c
Mean	601.92	26.41	56.86	16.02	19.55	720.65
$LSD_{0.05}$[b]	129.10	11.09	18.30	6.06	10.37	154.83
% Total	83.53	3.66	7.89	2.22	2.71	100.00

Data are the average of triplicates.

[a] Phenolic groups were quantified as follows: ellagic acids as ellagic acid, anthocyanins as cyanidin-3-galactoside, hydroxycinnamic acids as *p*-coumaric acid, flavonols as quercetin-3-galactoside, and benzoic acids as gallic acid.

[b] $LSD_{0.05}$: Least significant difference at the 0.05 level.

The bottom and neck parts of 'Chambly' contained the highest level of anthocyanins (869.33 and 751.78 μg g^{-1}, respectively), while the neck and bottom parts of 'LL0220-10' had the lowest (464.00 and 435.99 μg g^{-1}, respectively). There were no significant differences between the parts of 'LL0220-10'.

The bottom and neck parts of 'Chambly' had the biggest concentration of flavonols (86.32 and 85.39 μg g^{-1}, respectively) when compared with 'LL0220-10' (26.78 and 31.76 μg g^{-1}, respectively). There were no significant differences between bottom and neck parts of each cultivar.

Bottom part of 'Chambly' contained the highest levels of hydroxycinnamic and benzoic acids (60.70 and 46.06 μg g^{-1}, respectively), which were significantly different from the levels in its neck. No significant differences were found between the bottom and neck parts of 'LL0220-10'. Ellagic acids were significantly higher in the neck of 'LL0220-10' compared to its bottom, but there was no significant differences observed between neck and bottom of 'Chambly'.

The total phenolics of the five groups were obtained by HPLC and ranged from 477.27-1079.45 μg g^{-1}. The highest total of phenolics was found in the bottom and neck parts of 'Chambly' (1079.45 μg g^{-1} and 901.88 μg g^{-1}, respectively). There were no significant differences between the neck and the bottom parts of 'LL0220-10'.

The two strawberry cultivars differed in firmness, which was determined instrumentally (Table 1). Compared with 'Chambly', 'LL0220-10' was firmer and had a better shelf life, 3 versus 2 days.

The diversity of the chemical components confers the flavour, taste and aroma, to the strawberry. Good flavour is mainly obtained when high sugar and, especially, acid contents are at their optimum, (Kader 1991). In our experiment, SSC and TA varied, depending on strawberry part (Table 1). The whole fruit of 'LL0220-10' had the highest SSC, the whole fruit of 'Chambly' had the second highest SSC, and the lowest SSC was in the neck of 'LL0220-10'. The TA was the highest in the whole fruit of 'LL0220-10' and lowest in its bottom part. The highest pH values were found in the whole fruit and neck of 'Chambly' (4.00 and 3.90, respectively).

4. Discussion

Grey mold development is one of the principal causes of diseases at postharvest, which contributes mainly to shorter shelf life of strawberries. Strawberry petals are an important inoculum source of grey mold during flowering and fruit development (Boff, Kraker, Gerlagh, & Kohl, 2003). Infection remains underlying until fruits maturity and development is abundant which results in fruit rotening with much sporulation of the pathogen involved, *Botrytis cinerea* (Kovach, Petzoldt, & Harman, 2000). This pathogen is also responsible of significant losses at shipping, and for marketable fruits (Ceponis, Cappellini, & Lightner, 1987). Besides fungicides, many biological controlling agents may be effective in reducing disease caused by grey mold on strawberries (Guinebretiere, Nguyen-The, Morrison, Reich, & Nicot, 2000). However, these methods have some disadvantages for fruit yield or quality, and thus selecting and breeding resistant cultivars is the primary action for fruit producers.

Strawberry is a good source of natural antioxidants whose extracts exhibit high enzymatic activity against free radicals and for oxygen detoxification (Wang, Feng, Lu, Bowman, & Ding, 2005). Numerous studies have concluded that the antioxidant properties of constitutive and induced phenolic compounds provide good defence plant tissue against pathogen intrusions (Nicholson & Hammerschmidt, 1992; Prusky, 1996). Hébert, Gauthier and Gosselin (2001), and Hébert et al. (2002) used a similar method to that of Jersch et al. (1989) to find out that aqueous extracts of the unriped fruit of 'Chandler' had an unequivocal antifungal action against *B. cinerea* mycelial growth and conidial germination. In strawberry cultivars, the main phenolic components having antioxidant properties are: ellagic and gallic acids, anthocyanins (cyanidin-3-glucoside, pelargonidin-3-glucoside), catechin, and flavonols (quercetin-3-galactoside) which confers a better resistance to *B. cinerea*. A drop in strawberry disease resistance is associated with a reduction of specific proanthocyanins having antifungal activity during the development of the fruit, and the greatest antifungal activity is found in achenes at the green stage I of the fruits extracts, especially in achenes (Terry et al., 2004).

In this research, the highest total of phenolics was found in the bottom part of 'Chambly' in relation to its neck, whereas for 'LL0220-10' there were no obvious differences. Anthocyanins are the predominant group of unique phenolics in the anthocyanidins group responsible for the red colour of the strawberry flesh (Wang, Zheng, & Galletta, 2002). However, in the present work, we did not find correlations between individual PC and TAC, similar conclusion to that of Meyers, Watkins, Pritts and Liu, (2003). In previous studies, it was noted that there is positive or negative between free phenolics and TAC, in many kinds of fruits (Kalt et al., 2003; Rekika et al., 2005; Xie et al. 2013). Macheix, Fleuriet and Billot (1990) reported that strawberries contain plenty of different phenolic compounds, which differ depending on the cultivar. Furthermore, it has been noted that relative proportions and differences of PC, within the profiles, could afterwards produce multiple diversities in antioxidant activity (AA) or other bioactivities.

Ellagic acid has a negative impact on mycelial growth and germ tube elongation at low concentration (36 ppm), whereas it promotes them when high (Tao et al., 2010). Hébert et al. (2002) reported a better resistance level to grey mold growth using extracts of 6 strawberry cultivars, but it did not mention which were the phenolic compounds or mixes responsible for the inhibition of *B. cinerea* growth. As for conclusions by Tao et al. (2010), in this exercise, we found that there is a negative correlation between shelf life and phenolic compounds (data not shown). Strawberry flavour derives from tastes and aromas of many chemical components which interact together. High sugar and relative high acid content is required for good flavour (Kader, 1991). In this present study, even though 'LL0220-10' was firmer and had a shelf life that was 1 d longer compared with 'Chambly', the SSC and TA of the whole fruits of the two cultivars were similar.

In terms of anatomy, strawberry fruits with a raised neck and a reflexed calyx do not retain much water and have increased air circulation, features that may reduce infection by the pathogen. Literature reports that although strawberry fruits contain only 1% achenes based on fresh-weight, they contribute mostly to the AA and total phenolics (14% and 11%, respectively), and compared to immature ones, matured achenes contain more phenolics and have greater AA (Aaby, Skrede, & Wrolstad, 2005). In our study, the TAC of the bottom part of 'Chambly' was significantly higher in relation to its neck, which difference may be because of its higher achene content in that part.

In summary, the TPC and AA of strawberry may be due mainly to its genetic but also to their characteristical morphology and anatomy, which could also be in relation to its susceptibility to grey mold. Further investigation is then required for a better understanding.

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