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Folate, ascorbic acid, anthocyanin and colour changes in strawberry (*Fragaria* × *annanasa*) during refrigerated storage

Lisa Octavia, Wee Sim Choo*

School of Science, Monash University Malaysia, 47500 Bandar Sunway, Selangor, Malaysia

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Ascorbic acid
Cyanidin 3-*O*-glucoside
Cyanidin 3-*O*-rutinoside
Folic acid
5-methyl-tetrahydrofolate
5,10-methylene tetrahydrofolate
5-formyl-tetrahydrofolate
Pelargonidin-3-*O*-glucoside
Tetrahydrofolate

ABSTRACT

Colour and the composition of folate, ascorbic acid and anthocyanin of strawberry cv. Camarosa was monitored during refrigerated storage at 4 °C each day for 6 days. Folate, anthocyanin and ascorbic acid compositions were determined using High Performance Liquid Chromatography (HPLC) with UV-Vis detector. Five forms of folate: 5 methyl-tetrahydrofolate (THF) [65%], 10 formyl-folic acid (28%), 5 methylene-THF (4%), 5 formyl-THF (2%), and THF (<1%) and two forms of anthocyanins: pelargonidin 3-*O*-glucoside (97%) and cyanidin 3-*O*-rutinoside (3%) were identified in the fresh strawberries. The total folate content of the fresh strawberries determined using HPLC method was lower than that using microbiological assay. The ascorbic acid content in fresh strawberry was 57 ± 11 mg/100 g of fruit. The colour of the external skin of strawberries was measured using a Hunter colorimeter and showed fluctuation only for L* values (lightness), but a* (redness) and b* (yellowness) values remained constant during the refrigerated storage. There was no positive correlation between a* values and anthocyanin content. Strawberries should be consumed within a day or two after harvest since the reduction of these three bioactive compounds occurred even after a day of refrigerated storage.

1. Introduction

Strawberry (*Fragaria* × *annanasa*) has been cultivated in almost all regions of the world both for market and home use. Strawberry has a unique, highly desirable taste, colour and flavour and great amounts of bioactive compounds such as folate, which is known to have protective effects against cardiovascular disease, colorectal cancer and neural tube defects such as spina bifida (Stralsjo, Witthoft, Sjoholm, & Jagerstad, 2003a), vitamin C and other anti-oxidants including flavonoids which are capable of scavenging several different reactive oxygen species and may promote health benefits in liver protection, anti-cancer and coronary heart disease (Patil, Madhusudhan, Babi, & Raghavaro, 2009). Anthocyanins are flavonoids that are mainly found in strawberry and which are responsible for its attractive colour and indication of its ripeness level (Pinto, Lajolo, & Genovese, 2008). The quality of strawberry in the market is defined by its visual and internal characteristics such as colour, size, firmness, sweetness, acidity, aroma and the nutritional value, especially the antioxidant content (Shin, Ryu, Liu, Nock, & Watkins, 2008). Strawberry has a very short shelf-life due to fungal attack and natural ripening process that result in excessive texture softening. Therefore, good post-harvest handling and storage of the fruit should be practiced in order to preserve the quality of the fruit (Cordenunsi et al., 2005; Odriozola-Serrano, Soliva-Fortuny, & Martin-Belloso, 2010). Preservation methods such as low storage temperature, modified atmosphere packaging (MAP) or the addition of preservatives may be good ways to enhance the quality of fresh strawberry (Odriozola-Serrano et al., 2010).

Ascorbic acid can be easily exposed to enzymatic and oxidative

* Corresponding author.
E-mail address: choo.wee.sim@monash.edu (W.S. Choo).

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degradation. Various studies have found significant or insignificant decreases in ascorbic acid content in strawberry during storage at various temperatures (Shin et al., 2008; Cordenunsi et al., 2005; Cordenunsi, Nascimento, & Lajolo, 2003; Ayala-Zavala, Wang, Wang, & Gonzales-Aguilar, 2004). Studies on active and storage forms of folates have been done before, yet they are limited. Tetrahydrofolate (THF) and 5-methyl-THF were detected in strawberries (Vahteristo, Lehikoinen, & Ollilainen, 1997).

Anthocyanins in strawberry are susceptible to heat and light, resulting in colour deterioration of processed food containing strawberry, such as jam, during manufacturing and preservation. According to Zheng, Wang, Wang, and Zheng (2007), four major forms of anthocyanins (cyanidin 3-*O*-glucoside, cyanidin 3-*O*-glucoside-succinate, pelargonidin 3-*O*-glucoside and pelargonidin 3-*O*-glucoside succinate) were markedly affected by high oxygen treatment. In addition, the contribution of anthocyanins to the colour of the strawberry becomes the main parameter to determine the quality of the fruit (Lopez da Silva, Escribano-Bailon, Alonso, Rivas-Gonzalo, & Santos-Buelga, 2007; Nunes, Brecht, Morais, & Sargent, 1998; Crecente-Campo, Nunes-Damaceno, Romero-Rodriguez, & Vazquez-Oderiz, 2012). Studies investigating the colour changes of strawberry under various conditions have been carried out previously and significant or insignificant changes have been reported (Cordenunsi et al., 2005; Nunes et al., 1998; Zheng et al., 2007).

To date, there are no studies reporting on the effect of refrigerated storage (4 °C) on different forms of folate and anthocyanins in strawberry over a period of time. A temperature storage of 4 °C was chosen because that is the most common temperature of domestic refrigerator compared to other low temperature studies. The objective of this study was to investigate the compositions of three important bioactive compounds (ascorbic acid, folate and anthocyanin) and colour in strawberries and their changes during refrigerated storage at 4 °C for 6 days.

2. Materials and methods

2.1. Raw materials and chemicals

Freshly harvested strawberries cv. Camarosa were obtained on the same day from Cameron Highland, Malaysia. Strawberry with its fruit entirely red and approximately 6 °Brix of total soluble solids measured using a refractometer were used for all analyses. Sodium phosphate (monobasic and dibasic), ascorbic acid, metaphosphoric acid, acetic acid, 2-mercaptoethanol, hydrochloric acid, α-amylase from *Aspergillus oryzae,* carboxypeptidase G from *Pseudomonas* sp. and anthocyanin standards: pelargonidin 3-*O*-glucoside, cyanidin 3-*O*-glucoside and cyanidin 3-*O*-rutinoside were purchased from Sigma-Aldrich, St. Louis, U.S.A. Pronase protease from *Streptomyces griseus* and HPLC grade solvents: acetic acid, methanol, trifluoroacetic acid, acetonitrile and formic acid were purchased from Merck, Darmstadt, Germany. Folic acid *casei* medium (FACM) was purchased from Difco Laboratories, Detroit, U.S.A. *Lactobacillus rhamnosus* ATCC 7469 was obtained from American Type Culture Collection, Manassas, U.S.A. Folic acid, tetrahydrofolate (THF), 5-methyl-THF, 5,10-methylene THF, 10-formyl-folic acid and 5-formyl-THF were purchased from Schricks Laboratories, Jona, Switzerland.

2.2. Sample preparation

Fresh strawberries (200 g) were separated from their leaves, and then washed and dried using tissue papers. All analyses were conducted daily from day 0 (fresh strawberries) to day 6 of refrigerated storage at 4 °C. The storage time of 6 days was selected due to the severe bruising and tissue softening of the strawberries on the seventh day of refrigerated storage. All extracts were stored at −80 °C until analysis.

2.3. Ascorbic acid extraction

Extraction of ascorbic acid was carried out according to the method of van de Velde, Pirovani, Camara, Guemes, and Bernandi (2012) with some minor modifications. Strawberry (50 g) was homogenised using a Waring blender at 45000 rpm for 2 min. Approximately 6–7 g of the homogenised samples was added with 25 mL extraction solution containing metaphosphoric acid (30 g/L) and acetic acid (80 g/L). The mixtures were then sonicated for 15 min, followed by centrifugation at 12000 × g for 20 min at 4 °C. The supernatant (1 mL) was diluted with mobile phase (0.03 mol/L sodium acetate/acetic acid buffer) to achieve a final volume of 6 mL.

2.4. Folate extraction and deconjugation

Folate extraction and deconjugation was carried out in subdued light and all the glassware were wrapped with aluminum foil according to the method of O'Hare et al. (2012) with some modifications. Strawberries (50 g) were homogenised using a Waring blender at 45000 rpm for 3 min at 25 °C. The homogenised strawberries were collected and approximately 6 g of homogenised sample was treated with 20 mL buffer solution (0.1 mol/L potassium phosphate and 10 g/L ascorbic acid at pH 6.1). The mixtures were then boiled at 100 °C for 10 min. After cooling down, the homogenised samples were first incubated with 0.5 g of α-amylase (37 °C for 90 min), followed by 0.3 g of pronase protease E (37 °C for 90 min). Next, the samples were heated at boiling temperature in a water bath for 5 min. After immediately cooling down, the samples were centrifuged (2000 × g, 10 min, 4 °C). Supernatant (6 mL) was taken, added with 2 mg carboxypeptidase G, vortexed and incubated at 37 °C for 3 h, followed by centrifugation (2000 × g, 10 min at 4 °C).

2.5. Total folate content by microbiological test

The microbiological assay was performed based on the method of O'Hare et al. (2012). *Lactobacillus rhamnosus* was inoculated to 10 mL of sterile folic acid casei medium (FACM) (Difco Laboratories, Detroit, U.S.A.) supplemented with 0.3 mg/L folic acid and then incubated at 37 °C for 7 h. Next, 0.5 mL of the growth was subcultured into 100 mL of the same medium and incubated for 22 h. Immediately, the working inoculum was diluted with a normal sterile solution (1:40; v/v).

The diluted sample (50 μL) was transferred to a new test tube containing 2.5 mL assay buffer (0.05 mol/L sodium phosphate, 15 g/L ascorbic acid, pH 6.1) and 2.5 mL FACM. The working inoculum (50 μL) was then added to each tube of the mixture except for sample blanks, followed by incubation (37 °C, 22 h) in a shaking water bath. After incubation, the tubes were boiled for 5 min and vortexed. The absorbance measurement was carried out using a spectrophotometer (Lambda 25, Perkin Elmer UV/Vis Spectrometer, Waltham, U.S.A.) at 595 nm.

2.6. Anthocyanin extraction

Anthocyanin extraction was conducted based on the method of Lopez da Silva et al. (2007) with slight modifications. Approximately 80 g of strawberries was homogenised in 1 mL/L HCl in 100 mL methanol using a Waring blender at 45000 rpm for 2 min. The mixture was kept for 4 h at 4 °C. It was then filtered using a Buchner funnel under vacuum with glass microfiber filters GF/C