# Original article



# Organic and conventional strawberries: nutritional quality, antioxidant characteristics and pesticide residues

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# **Summary**

Introduction - Organic farming system may affect the food composition and produce healthy foods. The aim of this study was to compare the nutritional quality, antioxidant properties and pesticide residues of organic or conventional strawberries. Materials and methods - In a first experiment, organic and conventional fruits from the cvs. Camarosa and Albion were obtained directly from the farmers. Subsequently, 'Camarosa' was produced in organic and conventional systems, under controlled conditions. Pesticide residues were analysed in the second trial and the remaining parameters were evaluated in both experiments. Results and discussion - In the first experiment, the fruits of organic cv. Albion showed higher moisture (91.8%) and lower total solid (8.2%), and carbohydrate (5.7%) contents. 'Albion' fruits also showed higher total solid content than 'Camarosa' fruits in conventional farming system. Pesticide residues were not detected. Under controlled conditions, organic 'Camarosa' fruits had higher moisture (91.5%) and ash (0.4%) contents, whereas conventional strawberries had higher soluble solids (8.5 °Brix), proteins (0.9%) and anthocyanins (17.7 mg 100 g-1). Residues of azoxystrobin, lambda-cyhalothrin and thiamethoxam were detected at values below the limit of detection (< LOD) in all organic samples, and below the limit of quantification (< LOQ) in conventional strawberries. Conclusion - Organic and conventional production systems do not promote any expressive difference in the nutritional quality or antioxidant properties of strawberries, although the organic farming produced pesticide-free fruits.

#### Keywords

Brazil, strawberry, *Fragaria* × *ananassa*, agroecosystems, fruit quality, food safety

## Résumé

Fraises biologiques et conventionnelles: qualité nutritionnelle, propriétés anti-oxydantes et résidus de pesticides.

Introduction - Le système d'agriculture biologique peut affecter la composition des aliments et produire des aliments sains. Le but de cette étude était de

# Significance of this study

What is already known on this subject?

 Organic farming may affect the food composition and produce healthier foods than the conventional system, characterized by intensive use of chemical products.

What are the new findings?

 Organic farming produced pesticide-free fruits, but did not imply on substantial changes in the nutritional quality or antioxidant properties of strawberries.

What is the expected impact on horticulture?

 It is expected to encourage the organic farming system in order to produce healthy and pesticide-free strawberries.

comparer la qualité nutritionnelle, les propriétés anti-oxydantes et les résidus de pesticides des fraises produites en système biologique ou conventionnel. *Matériel et méthodes* - **Dans une première expérience**, les fruits biologiques et conventionnels des cvs. Camarosa et Albion ont été obtenus directement auprès des agriculteurs. Par la suite, les fraises 'Camarosa' ont été produites en systèmes de culture biologique et conventionnel, en conditions contrôlées. Les résidus de pesticides ont été analysés dans le deuxième essai et tous les autres paramètres ont été évalués dans les deux expériences. Résultats et discussion - Dans la première expérience, les fruits biologiques du cv. Albion ont présenté des teneurs en eau (91,8%) plus élevées, et des teneurs en matières solides totales (8,2%) et en hydrates de carbone (5,7%) plus faibles. Les fruits d'Albion' avaient également une teneur en solides totaux plus élevée que ceux de 'Camarosa' produits en système de culture conventionnel. Aucun résidu de pesticides n'a été détecté. En conditions contrôlées, les fruits biologiques de 'Camarosa' avaient des teneurs en eau (91,5%) et en cendres (0,4%) plus élevées, tandis qu'en conventionnel, les fraises contenaient plus de matières solubles (8,5 °Brix), de protéines (0,9%) et d'anthocyanes (17,7 mg 100 g-1). Des résidus d'azoxystrobine, de lambda-cyhalothrine et de thiaméthoxam ont été détectés, à des valeurs inférieures à la limite de détection (< LOD) dans tous les échantillons biologiques et inférieures à la limite de



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quantification (< LOQ) dans les fraises conventionnelles. Conclusion – Les systèmes de production biologiques et conventionnels ne favorisent pas l'expression de différences de qualité nutritionnelle ni de propriétés anti-oxydantes des fraises, même si l'agriculture biologique produit des fruits sans pesticides.

#### Mots-clés

Brésil, fraise, *Fragaria* × *ananassa*, agroécosystèmes, qualité du fruit, santé des aliments

#### Introduction

Strawberries (*Fragaria* × *ananassa* Duch.) are a commercially important food commodity, commonly consumed fresh or processed worldwide, and are appreciated by consumers, particularly for their attractive sensory characteristics, including aroma, flavor and color (Kovačević *et al.*, 2015). It is a low-caloric fruit, rich in fiber, vitamin C, folic acid, and bioactive compounds, such as anthocyanins and other flavonoids and antioxidants with relevant biological activities for human health (Fernandes *et al.*, 2012).

In Brazil, Espírito Santo State, strawberry cultivation has increased over the years and has played an important socio-economic role, mainly due to the increased income of small farms and the establishment of workers in rural areas. According to Costa *et al.* (2015), approximately 7,200 t were produced per year in 2012, involving more than 240 ha and generating approximately 2,500 direct jobs.

The Brazilian strawberry production is mainly derived from conventional farming system, characterized by intensive use of chemical products (Costa et al., 2015), generating residues, which may accumulate in natural and agricultural ecosystems depending on climatic, biological and edaphic factors (Camargo et al., 2009). Considering the potential hazardous effects of such farming system to the environment and human health, an environmental friendly approach, towards Agroecology, Sustainable Agriculture and Organic Farming has been proposed as an alternative farming system. The technologies adopted in this farming system aim to fulfil environmental, economic and social sustainability requirements, by reducing machinery, chemical fertilisers and pesticides (Goodman et al., 1992). The farming system, plant cultivar, type of soil, and climate among other factors may affect the chemical and physical properties of foods (Costa et al., 2015).

Studies investigating the effect of the agricultural farming system on the content of nutrients, bioactive compounds and toxic elements are scarce. Pires *et al.* (2015) did not find a clear superiority of organic fruits concerning mineral

composition. In their study, organic mango contained higher amounts of Mg and K, and lower content of Cr than conventionally grown fruits. Organic persimmon contained higher amounts of Cu and Zn, and lower content of K, Mg, P, and Na than the conventional fruits. Conventionally grown acerola contained higher amounts of Ca, Fe, Mn, Mo, Al, and Ni than the organic one, and the contents of Mo and Al were higher in organic strawberry when compared to conventional fruits. Peng et al. (2011), analysing strawberries from organic and conventional farms in USA, verified that fruits from organic culture contained significantly higher antioxidant capacities and flavonoid contents than those produced from conventional culture. However, Hargreaves et al. (2008) compared the effects of organic and inorganic fertilizer on the contents of sugar, macro- and micro-nutrients, and total antioxidant capacity, and did not find any increment on the quality of the strawberries.

Moreover, studies assessing pesticide residue in food products reported that controlled experiments, with adequate agronomic management, show contaminants in foods within the maximum residue limits (MRL) (Fernandes *et al.*, 2011). Conversely, the foods purchased in the retail market, which are available to the population, showed high values of pesticide residues (Phopin *et al.*, 2017), that are, in some cases, not allowed for the crop or banned in the country, indicating unsuitable farming management practices.

Due to the scarcity and inconsistency of results concerning the differences between the organic and conventional fruits, the current study was developed aiming to investigate the nutritional quality, antioxidant characteristics and pesticide residues of strawberries grown under these production systems. The study consisted on two experiments conducted in the central highlands of Espírito Santo, Brazil. It must be emphasized that the experiments were conducted under tropical conditions, not in the usual environment, temperate and subtropical, of this fruit. The first experiment compared the composition of organic and conventional strawberries grown by the local farmers and the second experiment was carried out under controlled conditions.

#### Materials and methods

Experiment 1 - Strawberries cultivated under organic and conventional farming systems

#### Sample collection

Strawberry fruit samples (*Fragaria* × *ananassa* Duchesne) of cvs. Camarosa and Albion grown by farmers in the central highlands of Espírito Santo under organic and conventional systems were collected directly from strawberry producers that previously had farming systems established

**TABLE 1**. Farming practices and inputs used in the strawberry crop management of Experiment 1.

| Sites | Latitude    | Longitude   | Altitude<br>(m) | Farming systems | Cultivars                  | Sowing periods         | Harvest dates                         | Pesticides  |
|-------|-------------|-------------|-----------------|-----------------|----------------------------|------------------------|---------------------------------------|---|
| 1     | 20°29'38" S | 41°04'32" W | 1,145           | Conventional    | 'Camarosa'                 | April 2012             | 04/9/2012<br>06/9/2012                | Procymidone,<br>Fluazinam                                 |
| 2     | 20°27'41" S | 41°04'39" W | 1,039           | Conventional    | 'Camarosa' and<br>'Albion' | April and<br>June 2012 | 26/9/2012<br>10/10/2012<br>25/10/2012 | Fluazinam,<br>Azoxystrobin,<br>Abamectin,<br>Fenpropatrin |
| 3     | 20°23'31" S | 41°01'54" W | 1,095           | Organic         | 'Albion'                   | June 2012              |                                       | -   |
| 4     | 20°18'35" S | 41°03'48" W | 954             | Organic         | 'Camarosa'                 | April 2012             |                                       | -   |

as agricultural practices for food production and an income source. The area is situated  $20^{\circ}22'14''S$  and  $41^{\circ}03'41''W$ , at an altitude of 940 m. The mean high temperature is between 26.7 and 27.8 °C and the low temperature between 8.5 and 9.4 °C.

The farmers were interviewed to collect data on farming practices and inputs used in their crop management (Table 1). The organic farming systems were certificated and validated by the authorized inspection agencies, according to internationally recognized standards enforced by the Brazilian legislation Law 10,831 of December 2003. In both cases, organic and conventional farming systems, the local farmers had received technical advises to follow the recommendations from INCAPER (Souza and Resende, 2006; Instituto Capixaba de Pesquisa, 2014; Balbino, 2006), as described in Experiment 2.

#### Fruit harvest and storage

The strawberries were selected considering the uniformity of the fruit ripeness (approximately 80%), color and absence of bruising and diseases. Following harvesting, the strawberries were homogenised in a home blender, packed in polyethylene bags and frozen at -18  $^{\circ}$ C.

Approximately 30 fruits were collected in 3 replications. All analyses were performed in triplicate. The data were submitted to Analysis of Variance (ANOVA), followed by Tukey test (at 5% probability) when applicable.

# Experiment 2 – Strawberries cultivated under controlled organic and conventional cropping systems

#### Experimental design

The experiment was performed using cv. Camarosa produced under controlled, organic and conventional cropping systems, in the experimental farm of the Capixaba Research Institute (INCAPER, Brazil), located in the same central highlands of Espirito Santo close by the site of Experiment 1 (20°22'17"S, 41°03'40"W, 950 m altitude). The cultivations were conducted in two adjacent areas separated by a plastic panel to avoid chemical and biological interferences. The organic cultivation occurred in a lot area managed for 22 years at the Agroecology References Unit of INCAPER and followed the principles of Agroecology, according to the Brazilian legislation (Law 10,831 of December 2003) for organic farming systems. The other cultivation followed the management practices for the conventional farming system, according to Souza and Resende (2006). The organic and conventional cropping systems are summarized in Table 2.

The experiment was conducted in a total experimental area of 140 m<sup>2</sup>. Each cropping system had 70 m<sup>2</sup> with

 $7\times10~m^2\text{-beds}.$  The five central beds of each system were used to sample fruits for analysis. The plants were spaced 0.35 m between rows and 0.3 m between plants in a row, distributed triangularly in the bed, and totalled 100 plants per plot with 10 m² of total usable area. Borders with a strawberry bed were installed laterally to the beds assessed in each cropping system.

Other management practices were similar in both cropping systems: low tunnels suitable for strawberry were installed to reduce leaf wetness; regular irrigation was performed using a hose over the tarpaulin; and weeding was performed manually when necessary.

#### Sample collection

A randomised block experimental design, with five collection replicates, was used in each cropping system. Each sample consisted of approximately 30 strawberries harvested from the identical bed, and each bed was considered one replicate. The mean of five beds was used in all laboratory tests to assess the effect of farming system. In physicochemical analyses, it was performed five laboratory replicates.

The fruit samples were collected at five different periods between August and September 2013. After harvesting, the strawberries were processed as described previously.

#### Organic farming system

Fertilization at sowing of the organic strawberries was based on organic compost produced locally. Composting was performed using 'Cameron' grass residues (produced in forage grass plantations adjacent to the area), crop residues from the production system itself (bean, maize and others) and coffee straw. The fertilizer applied at sowing was based on a soil analysis and followed the recommendations of IN-CAPER (2014), which indicated the use of 3.3 L m<sup>-2</sup> of compost broadcasted and incorporated by micro tractor rotary hoe prior to ploughing the beds. N- and K-enriched topdressing bio-fertilizers were applied starting at 30 days after sowing and bi-weekly until the mid-fruiting period. On average, the organic farming system consisted of: organic matter = 520 g kg $^{-1}$  soil; C/N Ratio = 16/1; pH in water = 7.3; macronutrient content in g kg-1 soil: N = 20.0; P = 11.9; K = 12.1; Ca = 48.3; Mg = 5.0; and micronutrient content in mg kg<sup>-1</sup> soil: Cu = 54; Zn = 188; Fe = 12; Mn = 793; B = 25. Pathogenic fungi Beauveria bassiana and Metarhizium anisopliae were used as biological pest control for plant disease management.

# Conventional farming system

Fertilization was performed based on a soil analysis following the recommendations of INCAPER (2014). Soil acidity correction was performed at a rate of 1.4 t ha-1 (140

**TABLE 2.** Farming practices of 'Camarosa' strawberries cropped in organic and conventional systems under the controlled conditions of Experiment 2.

| Latitude    | Longitude   | Altitude<br>(m) | Farming systems | Sowing date   | Harvest dates                       | Fertilization   | Pesticides/<br>Biological control  |
|-------------|-------------|-----------------|-----------------|---------------|-------------------------------------|---|--|
| 20°22'17" S | 41°03'40" W | 950             | Organic         | _ 18/04/2013  | 05/8/2013<br>12/8/2013<br>19/8/2013 | Composting: N-<br>and K-enriched<br>topdressing<br>biofertilizers | Pathogenic fungi: Beauveria<br>bassiana and Metarhizium<br>anisopliae                      |
| 20 22 11 0  | 33 10 17    | 330             | Conventional    | - 13,3 1/2010 | 26/8/2013<br>02/9/2013              | NPK formula<br>fertilizer<br>(20-0-20)                            | Azoxystrobin; Thiamethoxam;<br>Lambda-cyhalothrin;<br>Procymidone; Fluazinam;<br>Abamectin |



g m-2), and fertilization at sowing was performed by broadcasting and incorporation by micro tractor rotary hoe prior to ploughing the beds using the following fertilizers per m<sup>2</sup>: 1.5 L poultry manure, 226 g simple superphosphate and 10 g Fritted Trace Elements (FTE) - Micronutrients. NPK formula fertilizer (20-0-20) was applied as topdressing: 20 g m-2 at 60, 90, 120 and 150 days after sowing. Plant health management was performed using insecticides, miticides and fungicides according to the technical management recommendations for the crop typically adopted by conventional farmers (Balbino, 2006), as following: Azoxystrobin (pre-harvest interval = 1 day): 15 g 100 L-1 water; Thiamethoxam (preharvest interval = 1 day): 10 g 100 L-1 water; Lambda-cyhalothrin (pre-harvest interval = 3 days): 80 mL 100 L-1 water; Procymidone (pre-harvest interval = 1 day): 100 g 100 L-1 water; Fluazinam (pre-harvest interval = 3 days): 100 mL 100 L-1 water; and Abamectin (pre-harvest interval = 3 days): 30 mL 100 L-1 water.

#### Statistical analysis

The data were submitted to Analysis of Variance (ANO-VA), followed by Tukey test (5% probability) when applicable. It was not possible to distribute all the randomized repetitions, since they were different cropping systems (different environments). Because of the system-based design, the repetition was randomized only within each system. However, since there are homogeneous adjacent areas, the data variations are presumed negligible to affect comparative analyses.

#### Laboratory tests

The contents of protein, lipids, ash, carbohydrates, moisture, total solids, soluble solids, pH, total titratable acidity, vitamin C, anthocyanins, phenolic compounds and antioxidant activity were analysed in both experiments. The pesticide residue procymidone and lambda-cyhalothrin were screened in Experiment 1. Pesticide residues of azoxystrobin, lambda-cyhalothrin and thiamethoxam were analyzed only in Experiment 2.

#### Biochemical compounds

Protein (total nitrogen), ash, moisture, pH, total titratable acidity, and ascorbic acid (vitamin C) were determined according to AOAC (1995). The lipid analysis followed the Analytical Standards Methods of Instituto Adolfo Lutz (1985). The total carbohydrate content (TC) was calculated as the difference after measuring the moisture, ash, lipid and protein content. The total solid content (TS, in %) was calculated as the difference between the total sample and the moisture content of the same sample. The soluble solid content (SS) was measured by directly reading a benchtop refractometer (Atago, Japan), and the result was expressed as °Brix.

# Anthocyanins

The total anthocyanins of the strawberries were extracted using 70% ethanol acidified with HCl to pH 2.0 (Francis, 1982). Briefly, 5 g of the sample was added by 50 mL of 70% ethanol to perform the extraction in the dark for 16 h. The pigments were quantified according to Rodriguez-Saona et al. (1998), using a BEL Engineering spectrophotometer SF 2000 UV. Dilution of the original extract (5:10 v/v) was performed and the total anthocyanin content was expressed as pelargonidin-3-glycoside (433.2 g mol-1 molar mass). The molar absorption coefficient of 31,600 L cm-1 mol-1 was considered for the readings at a wavelength of 535 nm.

#### Total phenolic content

The total polyphenol index (TPI) determination was based on Singleton and Rossi (1965). Briefly, 0.6 mL of the anthocyanin extract was added to 3.0 mL of Folin-Ciocalteu reagent diluted in distilled water (1:10 v/v) and left to stand for 3 min in the dark. Subsequently, 2.4 mL  $\rm Na_2CO_3$  saturated solution (7.5% w/v) was added. Absorbance was measured at 760 nm in BEL Engineering spectrophotometer SF 2000 UV, following 1 h of incubation in the dark. The TPI was measured using a gallic acid standard curve (0–200 mg L-1), and the results were expressed as the gallic acid equivalent (mg GAE 100 g-1).

#### Antioxidant activity

The antioxidant activity was measured using the ABTS radical cation assay and the anthocyanin extracts. A 7-mM ABTS aqueous solution was added to a 2.45 mM potassium persulfate solution to form the ABTS•+ radical. This mixture remained in the dark at room temperature for 16 h. After the incubation period, the absorbance was corrected to 0.70 (±0.02) at 734 nm by adding 70% ethanol (Re *et al.*, 1999). Next, 0.5 mL of extract was added to 3.5 mL of the ABTS•+ radical solution, and a spectrophotometric reading was obtained after 6 min of reaction. The results were expressed in the Trolox equivalent (µM Trolox g-1).

#### Pesticide residues

The pesticide residues of Azoxystrobin – methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy] phenyl}-3-methoxyacrylate); Lambda-cyhalothrin – (R)- $\alpha$ -cyano-3-phenoxybenzyl(1S,3S)-3-[(Z)-2-chloro-3,3,3 trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate, (S)- $\alpha$ -cyano-3 phenoxybenzyl(1R,3R)-3-[(Z)-2-chloro-3,3,3-trifluoropropenyl]-2,2dimethylcyclopropanecarboxylate; and Thiamethoxam – 3-(2-chloro-1,3-thiazol-5-ylmethyl)-5-methyl-1,3,5-oxadiazinan-4-ylidene(nitro)amine were analysed in experiment 2. Procymidone (3-(3,5-dichlorophenyl)-1,5-dimethyl-3-azabicyclo[3.1.0]hexane-2,4-dione) and lambda-cyhalothrin were analysed in Experiment 1.

Stock standard solutions of 1,000 mg L-1 concentration were prepared by solubilising the azoxystrobin (99.9% m/m), thiamethoxam (99.7% w/w) (Sigma-Aldrich, Steinheim, Germany) and lambda-cyhalothrin (86.5% w/w) (Syngenta, São Paulo, Brazil), standards in acetonitrile (99.5% v/v) (Vetec, Rio de Janeiro, Brazil).

#### Pesticide extraction

The solid-liquid method of extraction with low-temperature partition (SLE/LTP) was used to extract the active ingredients in the strawberry matrix (Heleno *et al.*, 2014) by mixing 4.0 g strawberry pulp to 4.0 mL acetonitrile. The samples were stirred for 17 min at 25 °C and 200 rpm on a stirring table (Tecnal TE-420) and centrifuged for 4 min at 3,000 rpm in an Excelsa II centrifuge model 206 MP. The mixture remained at -20 °C for 10 h for phase separation by freezing the pulp. The organic extract was transferred into 2-mL vials for subsequent analysis.

# Chromatographic conditions

A gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) equipped with electron capture detector (GC/ECD) and AOC-20i auto injector was used. The detector temperature was maintained at 300 °C, nitrogen (99.999% purity) was used as the carrier gas, and injections were performed with 1:5 split.

FABLE 3. Mean values of pH, total titratable acidity (TA), total soluble solids (SS), proteins, lipids, ash, anthocyanins, phenolic compounds, antioxidant activity (AOA) and vitamin C of organic and conventional 'Camarosa' and 'Albion' strawberries. Data are mean values (n=3) from Experiment 1.

|               | ⊒<br>\$           | Δ                 | SS                | Proteins | Lipids            | Ash               | Anthocyanins      | Phenolics                     | AOA                | Vitamin C           |
|---------------|-------------------|-------------------|-------------------|----------|-------------------|-------------------|-------------------|-------------------------------|--------------------|---------------------|
| ractor levels | <u>5</u>          | (% citric acid)   | (°Brix)           | (%)      | (% wet basis)     | (%)               | (mg 100 g-1)      | (mg GAE 100 g <sup>-1</sup> ) | (µM Trolox g-1)    | (mg 100 g-1)        |
| Organic       | 3.49ª             | 1.09a             | 7.21a             | 0.89ª    | 1.25a             | 0.35a             | 18.07a            | 281.78ª                       | 73.10a             | 112.34ª             |
| Conventional  | 4.44a             | 1.14ª             | 7.65a             | 0.98ª    | 1.08ª             | 0.34ª             | 16.96ª            | 276.86a                       | 73.03a             | 97.93ª              |
| Means         | 3.97              | 1.12              | 7.43              | 0.935    | 1.16              | 0.34              | 17.51             | 279.32                        | 73.06              | 105.14              |
| 'Camarosa'    | 4.42 <sup>A</sup> | 1.12 <sup>A</sup> | 6.82 <sup>A</sup> | 0.88     | 1.26 <sup>A</sup> | 0.34^             | 18.6 <sup>A</sup> | 280.7 <sup>A</sup>            | 74.75 <sup>A</sup> | 98.97≜              |
| 'Albion'      | 3.51 <sup>A</sup> | 1.11≜             | 8.04^             | 0.99⁴    | 1.07≜             | 0.36 <sup>A</sup> | 16.44^            | 277.9 <sup>A</sup>            | 71.38 <sup>A</sup> | 111.32 <sup>A</sup> |
| Means         | 3.97              | 1.12              | 7.43              | 0.93     | 1.16              | 0.35              | 17.52             | 279.30                        | 73.06              | 105.15              |

Means in columns followed by the same lowercase or uppercase letter are not different at the 5% probability level by F-test

Separations were performed on a DB-5 capillary column (Agilent Technologies, Palo Alto, USA) with a 30-m length, 0.25-mm internal diameter and 0.10-µm film thickness. The following temperature programme of the column oven was used: initial temperature of 150 °C (2 min), temperature increasing rate of 40 °C min-¹ until 210 °C (2 min), followed by a 10 °C min-¹ rate until 250 °C (2 min), followed by a 20 °C min-¹ rate until 290 °C, and this temperature was maintained for 7 min. The injector temperature was maintained at 280 °C, and the carrier gas flow used was 1.2 mL min-¹. The injection volume was 1.0 µL, and the total analysis time was 20.5 min. The runs were managed using GC solution software (Shimadzu, Kyoto, Japan). The analytes were identified by comparing the peak retention time of the extracts from the samples with the standard retention time.

# Analytical procedure and values

The following parameters were validated in the analytical procedure to quantify the pesticide residues: selectivity, linearity, limits of quantification and detection, accuracy and precision. The method was applied to pesticide-free strawberry samples to evaluate selectivity. Aliquots of the identical sample were then fortified with azoxystrobin, lambdacyhalothrin and thiamethoxam and again submitted to the extraction and analysis method to determine the retention times of each substance. Linearity was evaluated using an analytical curve obtained by analysing sample extracts fortified with azoxystrobin, lambda-cyhalothrin and thiamethoxam at concentrations ranging from 0.3 to 0.6 mg kg-1, 0.25-1.00 mg kg<sup>-1</sup> and 0.075–0.200 mg kg<sup>-1</sup>, respectively (n = 18, 6 points in triplicates). The equations of the standard curve and coefficient of determination ( $R^2$ ), were generated from the injections of standard solutions of known concentrations. To assess precision, pesticide-free samples were fortified in six replicates at the following concentrations: azoxystrobin (0.45 mg kg-1), lambda cyhalothrin (0.25 mg kg-1) and thiamethoxam (0.10 mg kg-1), and results expressed the coefficient of variation. Accuracy was measured by recovery assays, in which known quantities of analyte were added in identical amounts to those utilized in the precision tests, also in six replicates. The results were expressed as recovery percentages. The limits of detection (LOD) and quantification (LOQ) were calculated in previous studies using the strawberry study matrix.

## Results and discussion

# Quality of strawberries produced under organic and conventional farming systems

The interaction between farming systems (organic and conventional) and cultivars ('Camarosa' and 'Albion') was not significantly different (P > 0.05), and no significant difference was observed between strawberries grown under organic and conventional farming systems or between the different cultivars regarding pH, total titratable acidity, soluble solids, proteins, lipids, ash, anthocyanins, phenolic compounds, antioxidant activity and vitamin C (Table 3). However, the interaction Farming × Cultivar regarding moisture, total solids and carbohydrates was significant ( $P \le 0.05$ ), and thereafter, the interaction was partitioned by comparing the means by Tukey's test.

The organic 'Albion' strawberries had higher moisture content than the conventional ( $P \le 0.05$ ), but no moisture difference was observed between organic and conventional



TABLE 4. Moisture (%), total solid (TSC, %) and mean carbohydrate (%) contents of organic and conventional 'Camarosa' and 'Albion' strawberries. Data are mean values (n = 3) from Experiment 1.

| Forming systems | Moistur    | e (%)    | TSC (      | %)       | Mean carbohy | /drates (%) |
|-----------------|------------|----------|------------|----------|--------------|-------------|
| Farming systems | 'Camarosa' | 'Albion' | 'Camarosa' | 'Albion' | 'Camarosa'   | 'Albion'    |
| Organic         | 92.14 aA   | 91.77 aA | 7.86 aA    | 8.22 bA  | 5.38 aA      | 5.74 ba     |
| Conventional    | 91.43 a A  | 89.49 ыв | 8.56 aB    | 10.51aA  | 6.11 aB      | 8.18 aA     |

Means followed by the same uppercase letter in rows, or the same lowercase letter in columns, are not different at the 5% probability, by Tukey's test.

'Camarosa' fruits (Table 4). The moisture content in fruit is directly related to its texture, which is one of the factors responsible for turgor and tissue firmness (Chitarra and Chitarra, 2005). The moisture content of strawberries may vary with the genetic characteristics of the plant, so, different cultivars may show different performances in the identical farming system, depending on the genotype × environment interaction.

A meta-analysis performed by Brandt et al. (2011), analyzing the content of secondary metabolites and vitamins in organic and conventional fruit and vegetables, showed that the average contents of vitamin C and all groups of secondary metabolites other than carotenes and the other 'non-defense compounds,' anthocyanins, tocopherols and volatiles, were higher in organic plant materials than in the corresponding conventional samples. Phenolic acids and total phenolics showed substantially higher contents in organically grown plants than in the conventional ones. In the current study, a significant difference ( $P \le 0.05$ ) in the total solid content only occurred between the different farming systems for cv. Albion (Table 4). The 'Albion' fruits had higher total solid values in the conventional farming system than 'Camarosa' ones, what emphasises the varied performances of different cultivars under an identical farming system. The mean carbohydrate values were significantly different  $(P \le 0.05)$ only between organic and conventional 'Albion' strawberries (Table 4).

Camargo et al. (2009) observed higher total soluble solids (SS), total titratable acidity (TA) and SS/TA ratio for cv. Sweet Charlie under conventional farming and higher amounts for 'Camarosa' under organic farming. In that study, the moisture content of 'Sweet Charlie' fruits on conventional system was significantly higher than that on organic system, what exhibited varied cultivar performances relative to the farming system for most variables studied.

Pesticide residues were not detected in any sample of Experiment 1. Procymidone and lambda-cyhalotrine pesticides were investigated due to the history of use in strawberry farms of this area and also because of the volatility of these compounds, which is relevant to the chromatographic method used.

Individual characteristics of fruits are related to environmental factors, and can be translated into different substances produced, which vary according to physiological or ecological factors (light, nutrition, water and plant health). Different cultivars may produce different amounts of secondary compounds under the same farming system.

# Quality of strawberries under controlled organic and conventional farming conditions

#### Nutritional, physicochemical and antioxidant characteristics

Analyzing the results obtained under the tropical conditions where the study was carried out, it was verified that no significant differences (P > 0.05) were found regarding pH, total titratable acidity, lipids, anthocyanins, phenolic compounds, antioxidant activity and vitamin C between organic and conventional farming systems (Table 5). Other antioxidant components as well as different analytical methods to evaluate the antioxidant activity, such as FRAP and DPPH (Moharram and Youssef, 2014) should be investigated in future studies. Significant differences (P < 0.05) occurred between organic and conventional farming for moisture, total solids (TS), soluble solids (SS), proteins, ash and carbohydrates. Organic strawberries were higher in moisture and ash, whereas conventional strawberries were higher in soluble solids and proteins (Table 6).

Studies comparing the nutrient composition of strawberries produced under conventional and organic systems are not conclusive, particularly regarding nutritional quality. For instance, Krolow et al. (2007) observed higher amounts of anthocyanins (36.29 mg 100 g-1), total soluble solids (7.2 °Brix) and TS/TA ratio (9.64) in organic compared to conventional strawberries (17.60 mg 100 g-1, 6.2 °Brix and 7.75, respectively). However, the ascorbic acid content was higher in conventional (56.50 mg 100 g-1) than organic strawberries. Lairon (2010) reviewed the data available from scientific literature and highlighted that organic plant products tend to have more dry matter, some minerals (Fe, Mg) and anti-oxidant micronutrients, such as phenolic compounds. The author also considered that organic agriculture has the potential to produce high quality products in improving the contents of antioxidant phyto-micronutrients under the best production conditions. Otherwise the antioxidant contents may vary significantly with major factors such as varieties, regions and locations, altitude, and particularly climate conditions (Lachman et al., 2009).

TABLE 5. Mean values of pH, total titratable acidity (TA), lipids, phenolic compounds, antioxidant activity (AOA) and vitamin C of organic and conventional 'Camarosa' strawberries produced under controlled conditions. Data are mean values (n = 5) from Experiment 2.

| Farming systems | рН    | TA<br>(% citric acid) | Lipids<br>(% wet basis) | Phenolics<br>(mg GAE 100 g-1) | AOA<br>(µM Trolox g-1) | Anthocyanins (mg 100 g-1) | Vitamin C<br>(mg 100 g-1) |
|-----------------|-------|-----------------------|-------------------------|-------------------------------|------------------------|---------------------------|---------------------------|
| Organic         | 2.93a | 0.86a                 | 0.89a                   | 372.51a                       | 314.24a                | 16.81a                    | 52.28a                    |
| Conventional    | 2.90a | 0.82a                 | 0.87a                   | 339.20a                       | 308.47a                | 17.72a                    | 45.48a                    |

Means followed by the same letter in columns are not different at the 5% probability by F-test.



**TABLE 6.** Mean moisture, total solids (TS), soluble solids (SS), proteins, ash and carbohydrates (Carb.) values of organic and conventional 'Camarosa' strawberries produced under controlled conditions. Data are mean values (n = 5) from Experiment 2.

| Farming systems | Moisture<br>(%) | TS<br>(%) | SS<br>(°Brix) | Proteins (%) | Ash<br>(%) | Carb.<br>(%) |
|-----------------|-----------------|-----------|---------------|--------------|------------|--------------|
| Organic         | 91.48a          | 8.53₺     | 8.13b         | 0.75b        | 0.43a      | 6.44b        |
| Conventional    | 91.03b          | 8.98a     | 8.50a         | 0.93a        | 0.35♭      | 6.80a        |

Means followed by the same letter in columns are not different at the 5% probability by F-test.

#### Pesticide analysis

- 1. Selectivity The retention time  $(t_R)$  peak for thiamethoxam, lambda cyhalothrin and azoxystrobin were 6.8 min, 10.8 and 11.1 min, and 15.2 min respectively. A comparison between extracts from the pesticide-free and pesticide-containing samples showed that no effect occurred on the retention time for any analyte.
- 2. Precision and accuracy The analytical procedure recovered 70 to 120% on average in each fortification level with a precision of CV < 20% (Brasil, Ministério da Agricultura Pecuária e Abastecimento, 2011) (Table 7).
- 3. Pesticide residues The pesticide residues of the organic samples were below the limit of detection (<LOD) (Table 8); namely, no residue was observed. The residue values of conventional strawberries were below the limit of quantification (<LOQ); thus, residues were observed but were unquantifiable, although the values of LOD and LOQ were close. The limits of quantification were lower than the Maximum Residue Limits (MRL) established by the National Health Surveillance (2006).

It is notable that the conventional management used in this experiment was based on the agronomic technical recommendations for quantity, concentration and pesticide application period allowed for the strawberry crop. Moreover, the fruits were harvested in the morning prior to applying any of the pesticides.

Azoxystrobin is a class III (slightly toxic) strobilurin fungicide. Strobilurins belong to a new class of systemic fungicides considered environmentally safe. Lambda-cyhalothrin is a non-systemic, class III toxic pyrethroid contact insecticide. Pyrethroids are synthetic insecticides derived from pyrethrins and are not persistent in the environment. Thiamethoxam is a neonicotinoid class III toxic systemic insecticide (Andrade *et al.*, 2011).

The physicochemical properties of pesticides, amounts, frequency of use, application method, biotic and abiotic characteristics of the environment and weather conditions affect the behaviour of the pesticides in the environment. Thus, a model that predicts the behaviour and interaction of these

**TABLE 7.** Statistical parameters, recovery and coefficient of variation (CV) calculated for the strawberry matrix. Data are mean values (n = 6).

| Pesticide compounds | Fortification concentration (mg kg-1) | Recovery<br>(%) | CV<br>(%) |
|---------------------|---------------------------------------|-----------------|-----------|
| Azoxystrobin        | 0.45                                  | 108.1           | 9.8       |
| Lambda-cyhalothrin  | 0.25                                  | 107.8           | 14.3      |
| Thiamethoxam        | 0.10                                  | 95.4            | 10.4      |

products in the environment cannot be designed (Spadotto, 2006; Ribas and Matsumura, 2009; Zavatti and Abakerli, 1999). Besides, degradation in the environment may occur through several processes, including photochemical processes; by microbial degradation; hydrolysis or oxidation-reduction; or the metabolism of plants and animals (Ribas and Matsumura, 2009). Once it is broken down, the pesticide may be fully or partially transformed into other molecules with higher or lower persistence and toxicity. Upon total decomposition, the final products are  $\mathrm{CO}_2$  molecules, water and mineral salts (Spadotto, 2006).

Some studies regarding pesticide residue shows contradictory results when compared with the results observed in the present study. Carvalho and Barbosa (2013) analyzed 136 tomato samples from different regions from the state of Minas Gerais, Brazil, between 2006 and 2008 and observed that 90 samples had dithiocarbamate and/or organophosphate pesticide residues. Approximately 60% of the contaminated samples had pesticide residues (methamidophos and ethyl chlorpyrifos) unauthorised for tomato. The percentage of samples contaminated with dithiocarbamate decreased in the last two years, and the concentrations were below the MRL (2.00 mg kg<sup>-1</sup>). Faria *et al.* (2009) observed that 95% of 55 samples of industrial strawberry pulp, in the state of Minas Gerais, Brazil, had pesticide residues, and 49% had unauthorised products, including acephate, captan, chlorfenapyr, chlorpyrifos, dimethoate, endosulfan, fenarimol, folpet, methamidophos, methyl parathion, prochloraz and tetradifon. Our results were obtained under controlled conditions and a possible difference between the present study and the others could be due to the non-control of the conditions of the other studies. Conversely, since Lairon (2010) reviewed data available from scientific literature about quality and security of organic food, he pointed that 94–100% of organic food did not contain any pesticide residue.

Guo *et al.* (2011) analyzed the organochlorine pesticide residues in bamboo sprout in Zhejiang, China, where three compounds were detected, hexachlorocyclohexane (HCH), 1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane (DDT) and pentachloronitrobenzene (PCNB). However, the majority

**TABLE 8.** Limit of detection (LOD), limit of quantification (LOQ) and maximum residue limits (MRL) for azoxystrobin, lambda-cyhalothrin and thiamethoxam pesticide compounds (Experiment 2).

| Pesticide compounds | LOD<br>(mg kg-1) | LOQ<br>(mg kg-1) | MRL*<br>(mg kg <sup>-1</sup> ) |
|---------------------|------------------|------------------|--------------------------------|
| Azoxystrobin        | 0.07             | 0.20             | 0.3                            |
| Lambda-cyhalothrin  | 0.07             | 0.22             | 0.5                            |
| Thiamethoxam        | 0.02             | 0.07             | 0.1                            |

<sup>\*</sup> MRL of strawberry crop established by ANVISA for the Programa de Analise de Resíduos de Agrotóxicos, PARA, Brazil.



of the samples (>82%) was safe for consumption, since the mean concentration of the residues did not exceeded the upper limit of 50 mg kg-1. There are studies corroborating data of the present study. Fernandes  $\it et~al.$  (2011), developed a survey study of strawberries produced in Portugal in the years 2009–2010, from organic farming and integrated pest management. Lindane and  $\beta$ -endosulfan were detected above the MRL in organic farming and integrated pest management. Other organochloride pesticides (aldrin, o,p'-DDT and their metabolites, and methoxychlor) were found below the MRL, and these residues decreased from 2009 to 2010.

Benbrook *et al.* (2009), referring to the study of Dangour *et al.* (2009), stated that organic fruit and vegetables might offer nutrient-related health benefits. Such nutritional benefits would be in addition to those that may come from reduced exposure to pesticide residues in conventional foods. Studies assessing pesticide residues in food products produced under controlled conditions shows that contaminants are within the MRLs. Furthermore, it is also important to note that problems related to pesticide use are not limited to food contamination but also include the poisoning of farm workers, water, soil and air.

#### Conclusion

The quality of strawberries produced under organic and conventional farming systems showed that the farming × cultivar interaction was not significant for most variables. A significant interaction occurred regarding moisture, total solid and carbohydrate. In both experiments, organic strawberries had higher moisture content and lower total solid and carbohydrate content than the fruits grown under conventional system and no significant difference was observed between the organic and conventional farming systems for strawberry quality regarding pH, total titratable acidity, lipids, anthocyanins, phenolic compounds, antioxidant activity and vitamin C. In the Experiment 2, the organic farming under controlled system showed also lower soluble solids and protein and higher ash contents than the organic fruits. In any case, the values, although significantly different, do not imply on a representative change in the fruit quality, since the variation of chemical compound contents were below 1%: protein (0.75-0.93%), ash (0.43-0.35%), moisture (91.48-91.03%), total solids (8.53–8.98%), and carbohydrates (6.4–6.8%).

Therefore, this study precludes the inference that different farming systems, organic and conventional, significantly affect the physicochemical, nutritional and antioxidant properties of strawberries.

Regarding the pesticide residues under controlled farming systems, no residues were observed on organic strawberries, and the residue concentrations observed on conventional strawberries were below the LOQ and could not be quantified. Thus, pesticide-free strawberries can be produced through organic farming when practiced in accordance with the established standards.

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