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Fruit responses to postharvest heat treatment time: characterisation of heat-treated strawberry (*Fragaria x ananassa*) cv. 'Candonga' fruits

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Abstract. Strawberries cv. 'Candonga' were heat-treated in an air oven (45°C) for 0.2 and 4 h, and then stored at 0°C for 2 days. One-way ANOVA revealed significant differences in terms of physical and chemical quality properties due to heat treatment time (P=0.000). In particular, as treatment time increased, strawberries showed a significant decrease of weight, firmness, redness (a*), yellowness (b*) and colour saturation (*chroma*). Additionally, the fruits were darker (lower L* value) after 2 h of treatment and lighter after 4 h (higher L* value). Among chemical quality properties, ph and soluble solids content (SSC) increased during treatments, whereas vitamin C content (TAA) decreased. After 4 h of treatment, total anthocyanins and total soluble phenolics (TSP) significantly decreased and increased, respectively. Principal component analysis (PCA) was executed on the correlation matrix of significant variables. Two principal components were extracted, explaining the 73.38% of the data variance. PC1 (60.15% variance) was associated with most of the physical and chemical variables, whereas PC2 (13.23% variance) was associated with fruit lightness. PCA was found to be of value in obtaining a visual representation of fruit samples based on heat treatment time.

Keywords: Heat treatment, principal component analysis, quality properties, refrigerated storage, strawberries

INTRODUCTION

Strawberries are an important crop worldwide. These fruits are considered healthy foods because they are rich in nutrients, such as anthocyanins, amino acids, and vitamins (Souci et al 2000). Nevertheless, strawberries are one of the most delicate and highly perishable fruits, being susceptible to mechanical injury, desiccation, decay and physiological disorders during storage.

Chemical treatments have been used to prevent insect attack and prolong postharvest shelf life. However, the use of chemicals has been minimised for food safety and environmental reasons. Therefore, many physical methods are being widely studied as a chemical alternative to extend fruit storability. An example of a physical method is heat treatment, often used in combination with refrigerated storage. It has been reported that the treatment at 45°C for 3 h in air can delay fruit ripening and reduce fungal attack in strawberries (García et al 1995; Civello et al 1997; Vicente et al 2002). Nevertheless, the effects of heat treatment on strawberry quality can be

contradictory, as influenced by a great number of factors, such as cultivar, ripening stage, heating procedures, and storage conditions (Lara et al 2006). Since fruit responses to physical methods could be diverse, the applicability of such treatments should be evaluated for each cultivar, even in terms of time of exposure.

The aim of the present study was to evaluate the effect of heat treatment time, in combination with refrigerated storage, on physical and chemical quality properties of strawberries cv. 'Candonga', for which no scientific investigation has been carried out. The analytical data of heat-treated samples were subjected to univariate and multivariate analysis to define the variables that mainly contribute to discriminating strawberries according to heat treatment time.

MATERIAL AND METHODS

Sampling, heat treatments and storage

Strawberries (*Fragaria x ananassa* cv. 'Candonga') for processing were selected according to their appearance (ripeness, size and colour), washed in distilled water and the peduncles removed. Then they were classified into three homogeneous groups and held in an air oven at 45°C for 0 (HTS-0h), 2 (HTS-2h) and 4 (HTS-4h) h. After the heat treatments, each group was stored at 0°C for 2 days, and then immediately analysed.

Physical analysis

The fruits of each group were individually weighed with an electronic digital balance (Model PM 400, Mettler, Greifensee, Switzerland). External colour was measured on three points of each fruit using a reflectance colorimeter (Model CR 200 Minolta, Ramsey, NJ, USA). Colour was recorded using the CIE–L^*, a^*, b^* uniform colour space, in which the L^* scale ranges from no reflection ($L^* = 0$, black) to perfect diffuse reflection ($L^* = 100$, white), the a^* scale ranges from negative values for green to positive values for red, and the b^* scale ranges from negative values for blue to positive values for yellow. Numerical values of a^* and b^* were used to calculate *chroma* ($[a^{*2}+b^{*2}]^{1/2}$), which indicates the intensity or colour saturation, and *hue* angle (arctangent[b^*/a^*]), where 0° = red–purple, 90° = yellow, 180° = bluish-green and 270° = blue (McGuire, 1992). Fruit firmness was measured using a penetrometer (Model FT 011, TR Scientific Instruments, Forlì, Italy) by applying a plunger of 6 mm in diameter.

Chemical analysis

The thalamus tissue of each fruit was separated manually from achenes and homogenised in a refrigerated blender at high speed for 2 min. The resultant homogenate was used for chemical measurements. Soluble solids content (SSC) was determined using a digital refractometer (Model PR, Atago, Tokyo, Japan) and results were expressed in percentage (%). One gram of homogenised tissue was poured into 9 ml of distilled water and pH of the filtered solution was measured using a standard pH meter (Model 744, Metrohm AG, Herisau, Switzerland), previously standardised to pH 4 and 7. Total anthocyanins and total soluble phenolics (TSP) were extracted overnight at 4°C from 1 g of homogenate with 9 ml of methanol containing 0.1% (v/v) of HCl.

After centrifugation at 1,500 x *g* for 10 min., the supernatant was filtered and its absorbance at 510 nm was measured. The amount of anthocyanins was calculated using the extinction coefficient (ε) equal to 3.6 x 10^{-6} 1 mol^{-1} m^{-1} (Woodward, 1972). Total anthocyanins content was expressed as mg of pelargonidin-3-glucoside (PGN) per g of tissue. For measuring TSP content, 1 ml of the same supernatant was added to 5 ml of 10% (v/v) of Folin-Ciocalteau reagent (Singleton & Rossi, 1965). After 3 min. at ~24°C (ambient), 4 ml of saturated solution of 7.5% (w/v) of Na_2CO_3 were added, and the reaction mixture was incubated for 2 h at the same temperature. The absorbance of the resulting blue colour was measured at 760 nm using a UV-Vis Spectrophotometer (Model Cary 50, Varian Inc., Walnut Creek, California, USA). Quantification was done on the basis of a standard curve of gallic acid and results were expressed as mg of gallic acid equivalents (GAE) per g of tissue. Total ascorbic acid (TAA) was measured by the classical titration method using 2.6-dichlorophenolindophenol solution in mg/g tissue (Charalambous, 1984; Miller, 1998).

Statistical analysis

All the variables were tested for normal distribution using the Shapiro-Wilk test (Shapiro & Wilk, 1965). One-way analysis of variance (ANOVA) was used to determine significant differences among groups due to heat treatment time. Means were ranked according to Tukey's HSD (honestly significant difference) multiple comparison test for responses that showed significance difference. The variables that showed high significance were used in the Principal Component Analysis (PCA) to identify a reduced number of principal components that sufficiently explain most of the information in the starting data. This analysis is a multilinear modelling method of pattern recognition, which shows the relationship between the objects (groups) on the basis of their distribution in the multidimensional space described from all the variables and also makes it possible to determine which variables are principally responsible for the separation of the objects.

RESULTS AND DISCUSSION

Physical analysis

The results concerning the effect of heat treatment time on physical quality properties of strawberries are shown in Table 1. Replicating previous studies (Vicente et al 2002; 2003), strawberry weight decreased during treatment time, with the HTS-4h group showing a lower weight, respectively, than the HTS-2h and HTS-4h groups. These results could be due primarily to different water loss among groups during treatments, which had a negative effect on fruit firmness: HTS-4h strawberries were softer than HTS-2h and HTS-0h strawberries, respectively. It has been reported that after the 3 h of hot air treatments (42, 45 or 48°C), strawberries cv. 'Selva' were slightly firmer than controls (Civello et al 1997; Vicente et al 2002), although no difference was found in earlier studies (Vicente et al 2003; 2005) and in other cultivars such as 'Chandler' (Yoshikawa et al 1992) and 'Pájaro' (Lara et al 2006). There were no significant differences detected among treatments after 7 days at 0°C, either (Vicente et al 2003). The lightness (L^* value) of the strawberries decreased from 0 to 2 h, as reported in previous studies (Vicente et al 2002; 2003). It has been reported that

after 7 days of refrigerated storage at 0°C, the decrease of *L** value was less evident in heat-treated fruits (Vicente et al 2003), although a previous study demonstrated that treated fruits showed higher *L** value after the same refrigerated storage (Vicente et al 2002). Our results show that HTS-4h exhibited a higher *L** value compared to the other groups, meaning that the fruit developed a lighter external colour after 4 h at 45°C and 2 days of refrigerated storage. The redness of the strawberry fruit (*a** value), as well as the colour saturation (*chroma* value), decreased during treatments, with fruits heated for 4 h showing lower values compared to the samples of other groups. These results indicated that the fruit surface became lower in pigment intensity during treatments. Yellowness (*b** value) decreased significantly after 2 h, but was similar in strawberries heated for 2 and 4 h. In contrast, *hue* angle was not affected by heat treatment time (P=0.456). Vicente et al (2002) reported that a significant increase of *hue* value could occur only after 7 days of refrigerated storage.

Table 1. Physical quality properties of heat-treated strawberries stored 2 days at 0°C.

Heat-treated groups	Weight (g)	Firmness (N)	External color				
			*L**	*a**	*b**	*Chroma*	*Hue* angle
HTS-0h	20.600 a	4.056 a	39.082 a	36.160 a	22.070 a	42.415 a	31.303
HTS-2h	19.123 b	2.563 b	36.070 b	31.327 b	18.666 b	36.543 b	30.683
HTS-4h	16.885 c	1.349 c	41.521 c	28.264 c	17.642 b	33.390 c	31.827
ANOVA (P=)	0.000	0.000	0.000	0.000	0.000	0.000	0.456

Means (standard deviation) in the same column with different letters are significantly different (P<0.05).

Chemical analysis

The data in Table 2 give the chemical quality properties of strawberries after heat treatments and storage at 0°C. The pH value increased significantly among treatments, with HTS-4h strawberries showing the highest value. These data conflicted with the findings of previous studies, in which no change of pH was observed after hot air treatment in strawberries cv. 'Selva' (Vicente et al 2002; 2003) and 'Pájaro' (Lara et al 2006). However, our results are more in line with those obtained by using hot water treatment (García et al 1995; Lara et al 2006), in which an increase of pH resulted from increased membrane permeability in heated fruit leading to enzymatic degradation of organic acids liberated from the vacuole. Soluble solids content significantly increased with heat treatment time: samples treated for 4 h showed the highest SSC content compared to other samples. These results are in agreement with García et al (1995) and Lara et al (2006) and are probably due to the increased activity of the invertase enzymes in the range of temperatures from 40 to 60°C (Ranwala et al 1992). Total

anthocyanins and total soluble phenolics increased from 0 to 2 h of treatment, but the differences were not significant. Similar results were found heating strawberries for 3 h at 45°C (Vicente et al 2002; 2003). However, Vicente et al (2002) reported that after 7 days at 0°C, the amount of anthocyanins was lower in heat-treated fruits. In our case, when heated for 4 h and then stored at 0°C for 2 days, strawberries showed less anthocyanins and more soluble phenolics compared to the other samples. The increasing treatment time decreases TAA content, which was lower in the HTS-4h groups than in the HTS-2h and HTS-0h groups, respectively. It has been reported that the vitamin C content can decrease as temperature increased (Wang & Camp, 2000), although other studies have reported that a temporary increase in the level of ascorbic acid can occur in response to heat treatment (Vicente et al 2006).

Table 2. Chemical quality properties of heat-treated strawberries stored 2 days at 0°C.

Heat-treated groups	pH	SSC (%)	Total anthocyanins (mg PGN/g)	TSP (mg GAE/g)	TAA (mg/g)
HTS-0h	3.461 a	9.973 a	0.201 a	1.968 a	0.590 a
HTS-2h	3.540 b	10.993 b	0.205 a	2.010 a	0.514 b
HTS-4h	3.631 c	11.663 c	0.170 b	2.576 b	0.392 c
ANOVA (*P*=)	0.000	0.000	0.000	0.000	0.000

Means (standard deviation) in the same column with different letters are significantly different ($P<0.05$).

Principal component analysis

From the correlation matrix of the original variables, Table 3, strong correlations are observed between physical and chemical variables. The highest positive correlations are shown by the pairs *a** – *chroma* (0.9412), firmness – *a** (0.8983), firmness – *chroma* (0.8408), firmness – *a** (0.8983), *b** – *chroma* (0.8224), firmness – TAA (0.8167), *a** – TAA (0.7775), *b** – *hue* (0.7589), weight – firmness (0.7546), *chroma* – TAA (0.7490), etc. In contrast, the pairs firmness – SSC (-0.8603), *a** – SSC (-0.8344), *chroma* – SSC (-0.7845), pH – TAA (-0.7761), SSC – TAA (-0.7572), *a** – pH (-0.7521), etc., showed the highest negative correlations. The complete data set obtained from the analysis of the physical and chemical quality properties of heat-treated strawberries was subjected to one-way ANOVA (Tables 1 and 2) to test the possible differences between the groups ($n = 3$) with the aim of selecting a small number of significant variables. Since heat treatment time had no effect on *hue* angle (P=0.456), this variable was discarded. The correlation matrix of the new data set selected was subjected to principal component analysis (PCA). The Scree plot (Figure 1) was used to identify the number of PCs to be retained in order to comprehend the underlying data structure (Jackson, 1991). The Scree plot shows a pronounced change of slope after the second eigenvalue; Cattell and Jaspers (1967) suggested using all the

Table 3. Correlation matrix of the physico-chemical variables.

Weight	Firmness	*L**	*a**	*b**	*Chroma*	*Hue* angle	pH	SSC	Total anthocyanins	TSP	TAA
1 (0.0000)	0.7546 (0.0000)	-0.3555 (0.0006)	0.6952 (0.0000)	0.3701 (0.0003)	0.6414 (0.0000)	-0.0989 (0.3538)	-0.5874 (0.0000)	-0.6698 (0.0000)	0.3663 (0.0004)	-0.4196 (0.0000)	0.6748 (0.0000)
	1 (0.0000)	-0.3314 (0.0014)	0.8983 (0.0000)	0.5118 (0.0000)	0.8408 (0.0000)	-0.0809 (0.4483)	-0.7456 (0.0000)	-0.8603 (0.0000)	0.3685 (0.0004)	-0.5487 (0.0000)	0.8167 (0.0000)
		1 (0.0000)	-0.2359 (0.0252)	0.1011 (0.3429)	-0.1259 (0.2369)	0.3065 (0.0033)	0.2899 (0.0058)	0.2395 (0.0230)	-0.3830 (0.0002)	0.4245 (0.0000)	-0.3792 (0.0002)
			1 (0.0000)	0.5826 (0.0000)	0.9412 (0.0000)	-0.0802 (0.4522)	-0.7521 (0.0000)	-0.8344 (0.0000)	0.3984 (0.0001)	-0.5028 (0.0000)	0.7775 (0.0000)
				1 (0.0000)	0.8224 (0.0000)	0.7589 (0.0000)	-0.5116 (0.0000)	-0.4861 (0.0000)	0.1528 (0.1504)	-0.2136 (0.0432)	0.4936 (0.0000)
					1 (0.0000)	0.2579 (0.0141)	-0.7387 (0.0000)	-0.7845 (0.0000)	0.3431 (0.0009)	-0.4379 (0.0000)	0.7490 (0.0000)
						1 (0.0000)	-0.0234 (0.8271)	0.0729 (0.4947)	-0.1288 (0.2264)	0.1263 (0.2355)	-0.0136 (0.8986)
							1 (0.0000)	0.7068 (0.0000)	-0.3336 (0.0013)	0.5317 (0.0000)	-0.7761 (0.0000)
								1 (0.0000)	-0.2547 (0.0154)	0.5169 (0.0000)	-0.7572 (0.0000)
									1 (0.0000)	-0.2894 (0.0057)	0.3507 (0.0007)
										1 (0.0000)	-0.4959 (0.0000)
											1 (0.0000)

Numbers in parenthesis are significance level (*P*=).

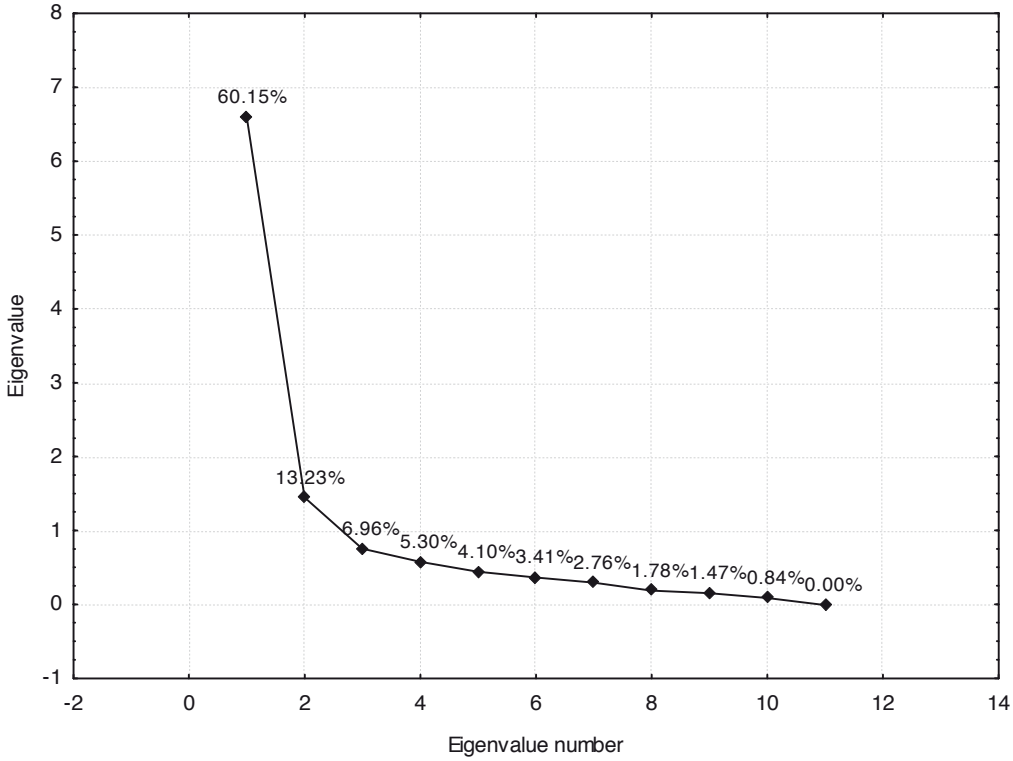

Figure 1. Scree plot of the characteristic eigenvalues.

PCs up to and including the first one after the break, so that two PCs were retained. They have eigenvalues greater than unity, thus respecting the Kaiser criterion (Kaiser, 1960). The two PCs explain 73.38% of the variance or information contained in the original data set. Projections of the original variables on the subspace of the PCs are called loadings and coincide with the correlation coefficients between PCs and variables. The loadings of the two retained PCs are presented in Table 4. The significant values were put in bold (the loadings were > 0.7000). PC1 explains 60.15% of the variance and is highly contributed by most variables. In particular, PC1 is positively correlated with firmness, redness (*a** value), colour saturation (*chroma* value), TAA and weight, and negatively with SSC and pH. These variables were demonstrated to be correlated (see correlation matrix, Table 3). PC2 explains 13.23% of the variance and is positively correlated with lightness (*L** value). A graphical representation of the loadings on the first two PCs is shown in Figure 2. As can be seen, total anthocyanins, yellowness (*b** value) and TSP are more distant from both the axes and closer to the centre of them (axes) compared to the other variables. This indicates their minor contributions to discriminate groups, confirming univariate results (see Tukey's HSD test, Table 1 and 2). Therefore, the application of PCA allows us to find out which variables contribute most to the differences among groups. The individual plan of the projection of the first two PCs (Figure 3) shows the excellent

separation among heat-treated strawberries. This demonstrates that the variables selected are able to explain the differences observed among the groups. In particular, a discrimination of the groups was essentially observed according to the principal component 1, since the scores for individual samples are spread along the PC1 axis. HTS-0h strawberries, characterised by high values of firmness, redness, colour saturation, TAA and weight, were on the positive side. HTS-4h strawberries, well-described by SSC and pH, showed negative scores, whereas the HTS-2h group was at the centre of the axis. Along the PC2 axis are spread samples according their *L** value. As can be seen, the HTS-2h samples showed negative values for this component, since they were characterised by a decrease of lightness compared to the other groups.

Table 4. Loadings of the significant physico-chemical variables on two first PCs.

Variable	Principal component	
	1	2
Weight	**0.7883**	-0.1246
Firmness	**0.9382**	0.0062
*L**	-0.3746	**0.7935**
*a**	**0.9359**	0.1258
*b**	0.6277	0.5749
Chroma	**0.9153**	0.3252
pH	**-0.8490**	-0.0140
SSC	**-0.8787**	-0.0921
Total anthocyanins	0.4573	-0.4660
TSP	-0.6207	0.3596
TAA	**0.8832**	-0.0488
Eigenvalue	6.6170	1.4548
Total variance (%)	60.1547	13.2251
Cumulative eigenvalue	6.6170	8.0718
Cumulative (%)	60.1547	73.3798

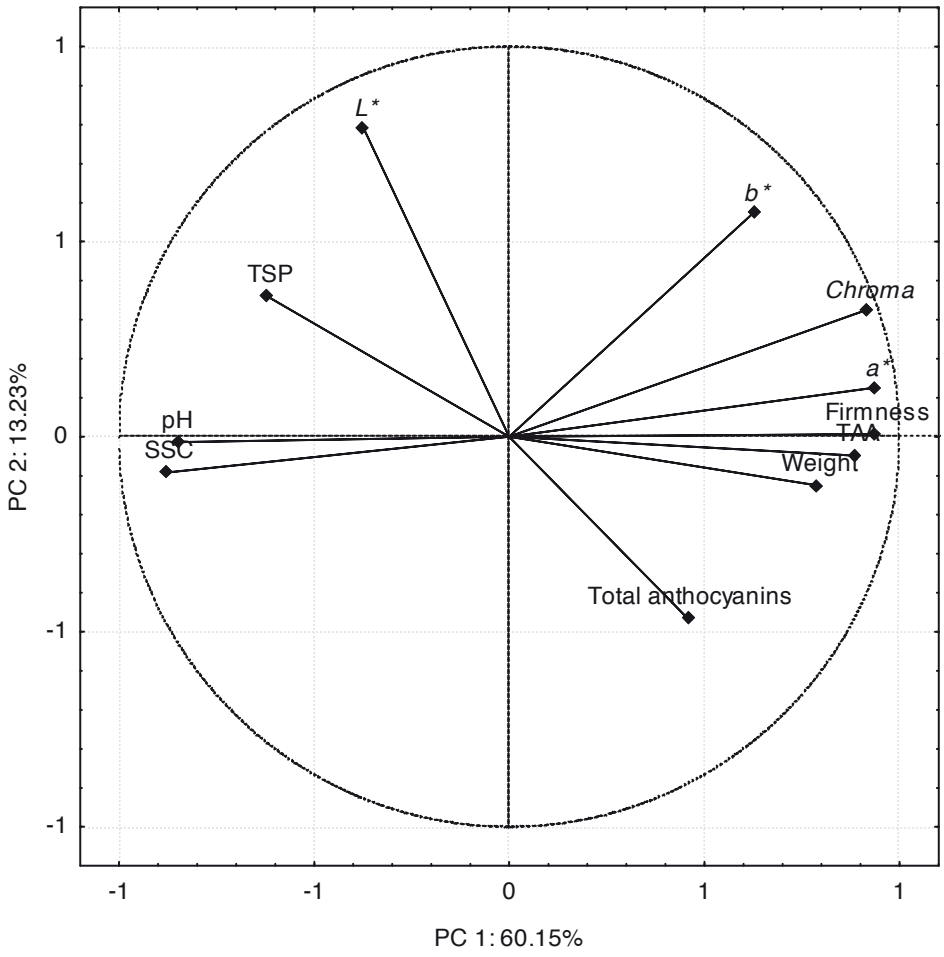

Figure 2. Plot of the first two PC weights of the variables.

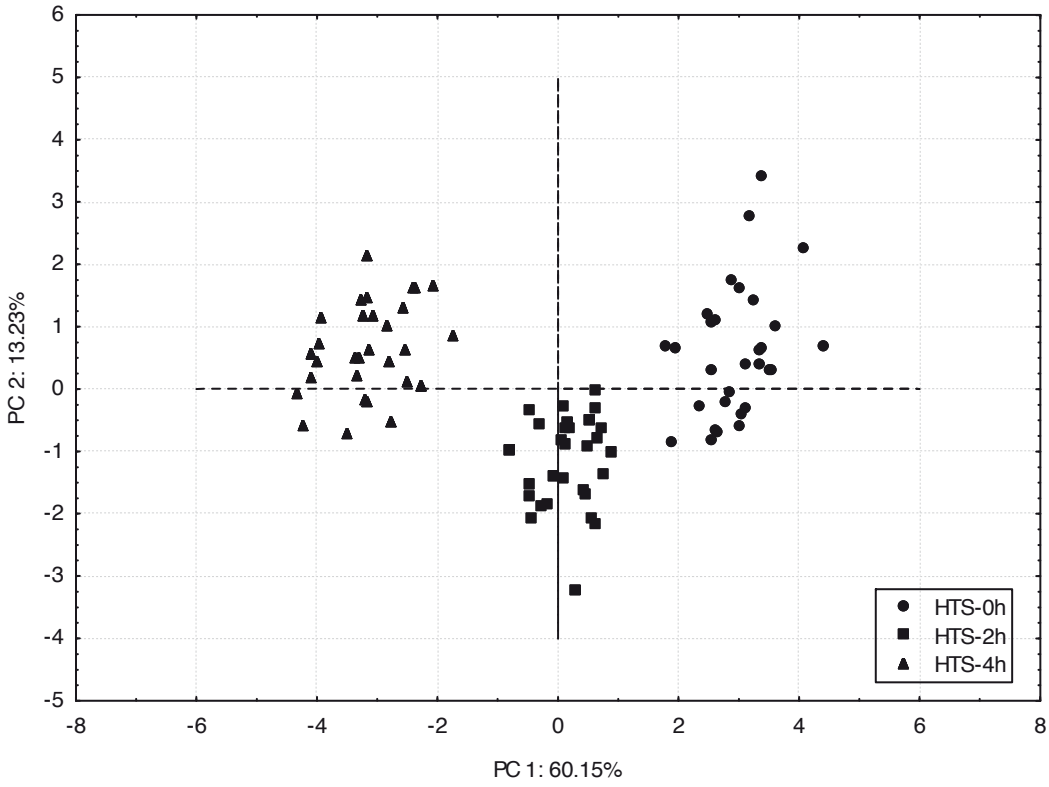

Figure 3. Plot of the scores of the samples on the first two PCs.

CONCLUSIONS

The results obtained in this research carried out with the cultivar 'Candonga' showed that heat treatment time, in combination with refrigerated storage, can affect the physical and chemical quality properties of strawberries. Weight, firmness, redness (*a**), yellowness (*b**) and colour saturation (*chroma*) were negatively affected, since their values decreased as treatment time increased. Additionally, fruits were darker (lower *L** value) after 2 h of treatment and lighter after 4 h (higher *L** value). No significant effect was found on *hue* angle. With regard to chemical quality properties, pH and soluble solids content (SSC) increased during treatments, whereas vitamin C content (TAA) decreased. After 4 h of treatment, total anthocyanins and total soluble phenolics (TSP) decreased and increased, respectively. However, further studies involving other strawberry cultivars need to be carried out to understand cultivar-related fruit responses to postharvest heat treatments. Principal component analysis (PCA) is a valuable tool to better detect these cultivar-related differences. In this study, the PCA executed on the correlation matrix of significant

variables extracted a lower number of 'latent' variables (principal components), which explains most of the variance of the original data set. Two principal components were extracted, explaining the 73.38% of the data variance. PC1 (60.15% variance) was associated with firmness, redness, colour saturation, TAA, weight (positive correlations) and with SSC and pH (negative correlations). PC2 (13.23% variance) was associated with fruit lightness. PCA allows us to obtain a meaningful characterisation of fruit samples based on heat treatment time and to find out which variables contribute most to their differences.

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