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Influence of the Storage Conditions on the Physicochemical Properties, Antioxidant Activity and Microbial Flora of Different Tomato (*Lycopersicon esculentum* L.) Cultivars

Ana F. Vinha[1,2], Sérgio V. P. Barreira[1], Ana Castro[1], Anabela Costa[2] & M. Beatriz P. P. Oliveira[2]

[1] FCS-UFP/Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Rua Carlos da Maia, Porto, Portugal

[2] REQUIMTE/Dep., Químicas Finas, Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira, Porto, Portugal

Correspondence: Ana F. Vinha, Faculdade de Ciências da Saúde, Universidade Fernando Pessoa, Rua Carlos da Maia, Porto, Portugal. Tel: 351-22-507-4630. E-mail: acvinha@ufp.edu.pt

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Abstract

This study intended to evaluate the influence of different postharvest cooling conditions in the physicochemical, antioxidant activity and microbiological contamination of tomato cultivars namely: Cereja, Chucha, Rama and Redondo. Pink fruits were kept for 15 days under three different storage temperatures (6ºC, 12ºC and 25ºC) during which their weight loss, total soluble solids, pH, color, titrable acidity, lycopene and ascorbic acid contents, total phenolics and antioxidant activity were evaluated every three days. Counts of colony forming units of coliform bacteria, yeasts and molds and the presence of *Escherichia coli*, were determined before and after 15 days of storage. All studied parameters revealed that temperature and storage duration caused statistically significant differences in nutritional values of every cultivar. It was observed an increase in the lycopene concentration and antioxidant activity with temperature and storage duration. The phenolic and ascorbic acid contents showed a slight increase during storage at every temperature. Microbial contamination was only found in cultivars that grew in direct contact with the soil (Chucha and Redondo) and bacterial population increased exponentially with storage temperature. Our results indicate that the ideal temperature to maintain optimal physiological, biochemical and microbiological profiles of the cultivars studied is 12ºC and that the cv. Rama fruit is the most suited for consumption *in natura.*

Keywords: tomato cultivars, physicochemical profile, microbiological profile, antioxidant activity, storage conditions

1. Introduction

Information about changes in bioactive compounds composition and their total antioxidant capacity during storage is required to offer consumers nutritionally sound fresh fruits. The fruit of the plant *Lycopersicon esculentum* L., commonly known as tomato, is one of the most widely consumed, being the second most important vegetable crop worldwide (Pantheen & Chen, 2010). Tomatoes are perceived by the general consumer as an antioxidant rich fruit that is highly perishable, having a short shelf life. This opinion is based on the observable alterations in the outer appearance of the fruit namely the rapid change in firmness, texture and color and the propensity to develop rottenness. This is why the ripening of tomato has been widely studied with the main objective to extend tomato consistency, color and shelf life (Moneruzzaman et al., 2009). Less obvious to the public are the changes that occur at the level of composition after the detachment of the fruit from the plant. These are undoubtedly the most relevant because they affect the nutritional value of tomatoes. Usually tomatoes are harvested at light red or breaker stage for distant transportation. The metabolism in tomato fruits continues even after their detachment from the plant. Besides the synthesis of pigments (e.g., lycopene) (Arias et al., 2000; Choudhary et al., 2009) the postharvest ripening stage has been associated with the production of flavor and aromatic compounds (Požrl et al., 2010) an increase in the ratio of citric to malic acid (Moneruzzaman et al., 2009; Saeed et al., 2009) and an increase in ascorbic acid content and total soluble solids (Toor & Savage, 2006). The antioxidant activity of tomatoes has also been reported to increase during storage (Toor & Savage, 2006). There is a growing interest in the beneficial health effects of tomato derived antioxidants (Carlsen et al., 2010; Korekar et al., 2011).

These chemical changes are expected to be influenced by the storage temperature (Gómez & Camelo, 2002; Sánchez-Moreno et al., 2006; Toor & Savage, 2006) and supposedly vary with the tomato cultivar (Anthon et al., 2011).

The study presented in this article was conducted in order to monitor the evolution of the physiologic parameters and antioxidant activity versus time of storage at different temperatures, for four different varieties of tomato fruits namely: Cereja, Chucha, Rama and Redondo. Since the deterioration of tomatoes during storage can also result from invasion by microorganisms (Babatola et al., 2008), counts of colony forming units of coliform bacteria, yeasts and molds and the presence of *Escherichia coli*, were determined before and after 15 days of storage.

2. Material and Methods

2.1 Material

Four tomato fruit cultivars Cereja, Chucha, Rama and Redondo, produced by the conventional way, in the North of Portugal were studied. Fruits were harvested when they reached the commercial point for "salad" i.e. suitable for table consumption. Fruits were selected by their firmness, absence of mechanical damage and visible decay.

Tomatoes were stored under refrigeration at different temperatures, 6°C, 12°C and 25°C with controlled relative humidity, between 90-95%. Fruits were packed in polystyrene trays and covered with polyvinyl chloride (PVC) film of 14 mm, for 15 days. Experimental tests were carried out in intervals of three days. The experiments were randomized, and six tests were performed for each analytical determination. Temperature and relative humidity were measured by an integrated probe (Thermohygrometer portable LabSet model HYGROPALM 0).

2.2 Reagents

Tillmans reagent (2,6-dichlorophenol), sodium carbonate, oxalic acid, ethanol, n-hexane, acetone and ascorbic acid were obtained from Sigma-Aldrich, St. Louis, MO, USA. Methanol, the Folin-Ciocalteu reagent, sodium hydroxide and gallic acid were purchased from Panreac Química Lda, Portugal. Milli Q filtered water (resistivity > 18 M cm) (Millipore, Bedford, MA) was used to prepare all aqueous solutions. Selective bile lactose agar with crystal violet and neutral red (VRBD), Plate Count Agar (PCA), TBX medium with chromogenic substrate, Sabouraud chloramphenicol agar and dehydrated Maximum Recovery Diluent necessary for microbiological culture media were purchased from bioMérieux, Portugal.

2.3 Physicochemical Analysis

A gravimetric assay was performed to evaluate the physiological tomato weight loss that was calculated by the difference between initial and final weight. A porcelain capsule containing 5g of fresh tomato was placed in a stove (WTC binder Klasse 2.0, Tuttlingen, Germany) at 105ºC ± 1ºC, followed by regular weighing up to a constant weight. Results were expressed in water percentage (%). pH values were measured in triplicate with a pH-meter (Microprocessor pH Bench-top HI 8417, Hanna Instruments). Total soluble solids (TSS) were quantified in a fruit puree using an Atago, NAR-3T refractometer, adjusted and calibrated at 20°C with distilled water and expressed as ºBrix, a rapid method to obtain an approximate value of total sugar content (ºBrix). A colorimetric method with a Color Quest II Sphere colorimeter (Hunter Lab) was used to determine color changes during storage, through the calculation of the °h (hue) of a portion of the each sample. The a^* (red-green) and b^* (yellow-blue) values were used to calculate the hue angle value, $\tan^{-1}$ (b^*/a^*), which provides information about the color index (color intensity) of fruit samples. The acidity was measured by the direct titration method with a strong titrant (NaOH = 0.1 M), according to the methodology described by the Association of Official Analytical Chemists (AOAC, 2005). A sample with 10 g of crushed fruits was dissolved in 90 mL of distilled water and shaken for 30 minutes. The sample was then titrated with standard sodium hydroxide (NaOH) using phenolphthalein (1%) as indicator, and results were expressed as citric acid grams per 100 g of sample.

2.4 Bioactive Compounds Quantification

2.4.1 Ascorbic Acid Assay

Ascorbic acid content was determined according to the previously described 2,6-dichlorophenolindophenol (DIP) method with some modifications (Vinha et al., 2012). Samples (5 g) of each tomato fruit were treated with 90 ml of oxalic acid (0.4%) for 1 hour and homogenized. The 2 ml of filtered extracts were diluted in 50 ml of distilled water and titrated with Tillman's reagent. Quantification was obtained from a standard curve based on the reduction of DIP and results were expressed as mg of ascorbic acid/100g.

2.4.2 Lycopene Quantification

Lycopene content was determined according to Sadler et al. (1990). A homogenized 5 g sample was added to a 50 ml mixture of hexane/acetone/ethanol (2:1:1, v/v/v) and incubated for 30 minutes. The absorbance of supernatant

(hexane layer) containing lycopene was examined using Beckman DU-64 spectrophotometer at 472 nm. Absolute hexane was used as blank. The amount of lycopene was then estimated using the equation:

$$Lycopene\,(\mu g / g) = \frac{A \times v \times 10^6}{3.450 \times W \times 100}$$

v is the amount of hexane (ml), W the weight of fruit sample (g), A the absorbance at 472 nm and 3.450 is the extinction coefficient.

2.4.3 Total Polyphenolic Content Assay

The method involving Folin-Ciocalteu reagent and gallic acid as standard was used for total polyphenolic determination, and carried out according to Zieliski and Kozowska (2000) with some modifications. Briefly, 5 g of fresh tomato fruits were homogenized (model F.60, Falc Instruments, Italy) in bi-distilled water (100 ml) kept at 40ºC for one hour and then filtered. The tomato fruit extracts were then resuspended in bi-distilled water and the supernatant (0.5 ml) was mixed with 0.5 ml of Folin-Ciocalteu's solution. The solution was homogenized for 3 minutes and 1 mL of saturated Na_2CO_3 was added. The solution was then incubated for 1 hour in the dark to obtain colour development, through the reduction of phosphomolybdic and phosphotungstic acids in an alkaline medium. Total phenolic content was based on a colorimetric method using Folin-Ciocalteau reagent. The absorbance readings were measured at 720 nm with an UV-VIS spectrophotometer (Shimadzu UV-2100), using gallic acid (GA) as standard ($y = 0.871x + 0013$, $r^2 = 0.999$).

2.5 Microbiological Analysis Control

Sixteen samples of each tomato cultivar, randomly chosen, were evaluated. Methods to determine microbiological contamination followed the recommended Portuguese Standard methods (NP, 1987; 1990; 2002) for the detection of mesophiles, total coliforms, *Escherichia coli,* molds and yeast. Colony forming units per gram (CFU/g) of fruit were determined. The standards and criteria for analyzing the results followed the legislation for fruits and vegetables. To ensure public health protection regarding total coliforms (CFU/g), grey molds and yeasts (CFU/g) s counts must be $< 10^2$, as is recommended and published by Reis, Finger, and Nery (2003). Counts above 10^4 CFU/g indicate potential contamination.

2.6 Antioxidant Activity Evaluation during Storage

A rapid, simple and inexpensive method to measure the antioxidant capacity of food involves the use of the free radical, 2,2-Diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$). $DPPH^{\bullet}$ is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. The radical scavenging activity of each tomato fruit extract was determined as previously described (Jang et al., 2007) with some minor modifications. Briefly, an aliquot of 1.5 ml of $DPPH^{\bullet}$ in MeOH (20 mg/l) was added to 0.5 ml of polyphenolics extract for 60 minutes in the dark. Absorbance (A) was measured every 10 minutes over a 60 minute time period at 517 nm using an UV spectrophotometer (Shimadzu, UV-2100 Series). Radical scavenging activity (RSA) was expressed as percentage of inhibition and calculated using the equation:

$$\%\,RSA = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

2.7 Statistical Analysis

A completely randomized design was used with six replications. Statistical analysis was performed using Prisma 5. Data of all analysis were expressed as mean ± standard error. Results were statistically evaluated by variance analysis (ANOVA) and statistical differences with p-values under 0.05 were considered significant. Tukey's test was performed to analyze differences among means. Hoteling's test was used for independent observations, to assess the differences between the profile dependence on physicochemical, bioactive compounds content, microbiological testing and antioxidant activity for each tomato cultivar studied. Pearson correlation tests ($p \leq 0.05$) were used to ascertain the existence of linear relationships between variables lycopene content/color, lycopene/antioxidant activity, total phenolic content/antioxidant activity and ascorbic acid/antioxidant activity.

3. Results and Discussion

3.1 Physiological Variation

The postharvest losses in terms of quality and quantity of food are a major problem all over the world. In the case of the tomato fruit significant losses are caused by inappropriate storage methods. The postharvest life of tomatoes usually does not exceed 2-3 weeks and is limited by physiological deterioration of the fruit related to over-ripening and pathogen infection which leads to decay. Many factors, such as cultivar variety, stage of maturity, size grading,

method of harvesting, handling, packaging and mode of transport, affect the storability of tomato fruits. Table 1 summarizes the results of the physiologic evaluation made on four Portuguese tomato fruit cultivars during 15 days at three different temperatures.

Table 1. Physiological characterization of the tomato fruits during storage at 6°C, 12°C and 25°C (± 1ºC) for 15 days. (pH) pH value; (ºh) color angle (ºh); (TSS) total soluble solids (ºBrix); (TA) citric acid content (mg/100g).

Cultivar		First Day	3rd Day	6th Day	9th Day	12th Day	15th Day
Storage Temperature 6ºC							
Cereja	pH	4.14±0.03^{aA}	4.14±0.02^{aA}	4.12±0.02^{aA}	4.10±0.05^{aA}	4.05±0.04^{bA}	4.01±0.02^{cA}
	ºh	60.1±1.7^{aA}	60.0±1.5^{aA}	59.7±0.1^{aA}	59.5±0.1^{aA}	56.1±1.2^{bA}	53.2±0.12^{cA}
	TSS	4.42±0.2^{aA}	4.41±0.1^{aA}	4.41±0.2^{aA}	4.42±0.2^{aA}	4.41±0.2^{aA}	4.41±0.09^{aA}
	CA	319.7±1.1^{aA}	319.6±0.2^{aA}	319.8±0.1^{aA}	318.5±0.8^{bA}	318.7±0.2^{bA}	318.9±0.9^{cA}
Chucha	pH	4.51±0.03^{aA}	4.52±0.08^{aA}	4.55±0.09^{aA}	4.49±1.23^{bA}	4.49±1.03^{bA}	4.51±0.81^{abA}
	ºh	50.9±1.7^{aA}	50.7±1.2^{aA}	48.7±0.09^{bA}	46.1±0.05^{bA}	43.1±0.06^{cA}	40.9±0.62^{dA}
	TSS	4.03±0.1^{aA}	4.03±0.8^{aA}	4.08±0.02^{aA}	4.02±0.1^{aA}	4.19±0.01^{bA}	4.15±0.04^{bA}
	CA	232.3±1.9^{aA}	232.0±0.1^{aA}	228.7±1.5^{bA}	225.9±0.02^{cA}	226.0±3.0^{cA}	225.9±3.0^{cA}
Rama	pH	4.41±0.06^{aA}	4.42±0.52^{aA}	4.42±0.03^{aA}	4.38±3.02^{bA}	4.35±1.99^{bA}	4.33±1.02^{bA}
	ºh	40.5±0.05^{aA}	40.5±0.02^{aA}	40.4±0.21^{aA}	40.5±0.08^{aA}	40.6±3.68^{aA}	40.5±4.96^{aA}
	TSS	4.35±0.36^{aA}	4.35±0.31^{aA}	4.34±0.96^{aA}	4.33±1.22^{aA}	4.32±2.09^{aA}	4.31±0.03^{bA}
	CA	249.5±0.0^{A}	249.6±1.9^{aA}	249.4±0.9^{aA}	248.1±2.6^{bA}	248.1±0.6^{bA}	248.2±0.9^{bA}
Redondo	pH	4.43±0.51^{aA}	4.45±0.05^{aA}	4.45±0.31^{aA}	4.45±0.02^{aA}	4.43±0.04^{aA}	4.41±0.08^{aA}
	ºh	50.2±0.52^{aA}	50.7±0.87^{aA}	49.7±3.41^{aA}	48.6±2.55^{bA}	46.5±0.05^{bA}	43.9±3.47^{cA}
	TSS	4.56±0.05^{aA}	4.56±0.54^{aA}	4.54±1.22^{aA}	4.47±2.74^{bA}	4.55±0.74^{aA}	4.52±0.31^{aA}
	CA	304.9±0.5^{aA}	304.8±0.0^{aA}	304.5±0.3^{aA}	304.7±0.4^{aA}	304.5±1.9^{bA}	304.5±2.6^{bA}
Storage Temperature 12ºC							
Cereja	pH	4.14±0.03^{aA}	4.15±0.89^{aA}	4.17±0.14^{bA}	4.18±0.06^{bB}	4.20±0.06^{bB}	4.21±0.06^{bB}
	ºh	60.1±1.54^{aA}	60.1±0.05^{aA}	58.7±2.73^{bA}	55.2±0.84^{cB}	54.2±1.23^{cdB}	51.2±0.08^{deB}
	TSS	4.41±0.02^{aA}	4.42±0.91^{aA}	4.42±0.08^{aA}	4.43±0.02^{aA}	4.43±1.23^{aA}	4.45±2.87^{bB}
	CA	319.7±0.9^{aA}	319.5±0.1^{aA}	319.6±.5^{aA}	319.8±0.1^{aB}	318.9±2.9^{bB}	319.9±2.6^{acB}
Chucha	pH	4.71±2.01^{aA}	4.52±0.08^{bA}	4.50±1.32^{bA}	4.49±0.76^{bA}	4.50±0.09^{bA}	4.51±2.61^{bA}
	ºh	50.9±0.99^{aA}	47.5±1.21^{bA}	41.2±3.64^{cB}	38.3±2.66c^{dB}	35.4±1.87^{dB}	32.9±0.34^{eB}
	TSS	4.03±0.02^{aA}	4.04±0.06^{aA}	4.06±0.21^{aA}	4.11±2.36^{bB}	4.13±1.99^{bB}	4.5±2.96^{cB}
	CA	232.3±0.1^{aA}	232.5±0.1^{aA}	232.8±0.1^{aB}	233.1±0.9^{bB}	233.2±2.6^{bcB}	233.2±1.4^{cB}
Rama	pH	4.41±0.02^{aA}	4.42±0.06^{aA}	4.43±0.02^{aA}	4.51±0.12^{bB}	4.56±2.96^{bB}	4.75±0.07^{bB}
	ºh	40.5±1.45^{aA}	40.5±0.99^{aA}	40.2±0.86^{aA}	40.1±0.15^{aA}	40.1±1.27^{aB}	39.9±0.03^{bB}
	TSS	4.35±0.06^{aA}	4.36±0.04^{aA}	4.34±0.08^{aA}	4.39±1.74^{bB}	4.41±2.61^{bcB}	4.39±0.08^{bdB}
	CA	249.5±0.1^{aA}	249.7±1.7^{aA}	248.6±2.5^{bA}	248.9±0.2^{bB}	247.8±0.1^{cB}	247.5±0.1^{cB}
Redondo	pH	4.43±1.22^{aA}	4.41±0.05^{aA}	4.42±1.41^{aA}	4.46±0.06^{bA}	4.45±0.07^{abB}	4.37±0.78^{cB}
	ºh	50.2±2.23^{aA}	49.9±0.78^{aA}	47.1±0.04^{bB}	44.9±0.09^{acA}	42.8±1.81^{dB}	42.01±0.71^{dB}
	TSS	4.56±0.10^{aA}	4.55±1.47^{aA}	4.51±0.08^{bA}	4.56±1.23^{abB}	4.30±0.05^{eB}	4.31±0.08^{cdB}
	CA	304.9±0.1^{aA}	304.5±0.1^{aA}	304.1±1.9^{bA}	304.3±0.1^{abA}	303.8±0.0^{abB}	303.9±0.8^{abB}
Storage Temperature 25ºC							
Cereja	pH	4.14±0.03^{aA}	4.21±0.02^{bA}	4.22±0.02^{cC}	4.17±0.05^{dC}	4.22±0.04^{eC}	4.21±0.02^{fC}
	ºh	60.1±1.73^{aA}	55.6±1.46^{bB}	55.7±1.02^{bB}	51.8±2.15^{cC}	50.3±0.94^{dC}	48.8±0.92^{eC}
	TSS	4.40±0.02^{aA}	4.37±0.05^{bB}	4.39±0.05^{acB}	4.40±0.11^{acdB}	4.41±0.15^{acdB}	4.44±0.83^{eB}
	CA	319.7±0.4^{aA}	315.7±2.3^{bB}	316.5±1.4^{cB}	316.7±1.4^{cdC}	317.3±1.7^{eC}	319.9±1.3^{fC}
Chucha	pH	4.70±0.08^{aA}	4.48±0.06^{bB}	4.54±0.03^{cB}	4.49±0.0^{5B}	4.54±0.11^{ceB}	4.68±0.09^{fB}
	ºh	50.9±1.30^{aA}	39.4±1.16^{bB}	35.8±1.46^{cC}	34.2±0.87^{dC}	31.7±1.09^{eC}	30.2±1.46^{fC}
	TSS	4.03±0.02^{aA}	4.13±0.05^{bB}	4.24±0.08^{cB}	4.31±0.05^{dC}	4.48±0.16^{eC}	4.56±0.19^{fC}
	CA	232.3±4.3^{aA}	234.8±2.1^{bB}	232.6±2.4^{cB}	234.3±1.4^{adC}	235.7±1.1^{eC}	236.4±0.9^{fC}
Rama	pH	4.41±0.04^{aA}	4.41±0.03^{aA}	4.85±0.09^{bB}	4.79±0.08^{cC}	4.86±0.07^{dC}	4.93±0.08^{eC}
	ºh	40.4±1.80^{aA}	40.4±0.09^{aA}	42.0±2.00^{aB}	41.2±1.17^{aB}	40.5±1.12^{aC}	39.4±0.83^{aC}
	TSS	4.35±0.05^{aA}	4.35±0.05^{aA}	4.46±0.07^{bB}	4.73±0.17^{cC}	4.52±0.23^{dC}	4.41±0.19^{eC}
	CA	249.5±3.8^{aA}	246.5±2.9^{aB}	249.5±3.8^{aB}	247.5±2.0^{aC}	248.7±2.6^{aC}	246.7±1.3^{aC}
Redondo	pH	4.43±0.06^{aA}	4.77±0.14^{bB}	4.56±0.13^{cB}	4.51±0.05^{cdB}	4.42±0.19^{eC}	4.39±0.07^{fC}
	ºh	50.2±2.50^{aA}	48.8±1.30^{aB}	43.2±3.70^{bC}	41.9±2.10^{cB}	40.5±0.79^{cdC}	40.8±0.67^{cdC}
	TSS	4.56±0.20^{aA}	4.33±0.10^{bB}	4.80±0.12^{cB}	4.05±0.04^{dC}	3.74±0.34^{eC}	4.01±0.41^{dfC}
	CA	304.9±3.6^{aA}	301.7±2.2^{aB}	304.2±3.4^{aB}	303.4±2.2^{aB}	304.3±1.8^{aC}	305.6±0.8^{aC}

*Values expressed as mean±standard deviation obtained from 6 measurements. For each cultivar, different lowercase superscripts indicate statistical significant differences caused by the storage time. Capital letters indicate significant differences related to different storage temperature.

The results reveal that the physicochemical profile of tomato fruits changes significantly over time and with the storage temperature as already reported by other researchers (Okolie & Sanni, 2012). Acidity and pH are two key parameters that ensure the quality of the fruit. The more mature the tomatoes the higher the pH. The acid content, on the other hand, has been reported to increase during ripening to the breaker stage and then decrease (Tang & Wang, 2003). The acid concentration is important because it affects the flavor of the fruit. According to the results presented in Table 1, all cultivars studied seem to suffer a pH increase until the 6th day of storage, followed by a decrease and subsequent increase. This variation is more pronounced the higher the temperature. A similar behavior was observed by Gómez and Camelo (2002) with reductions and consequent increases observed in the Diva tomato under storage conditions of 12ºC of temperature for 21 days. Batu (2004) explained the temperature dependence observed by a reduction on the metabolic processes when stored under low temperatures. Regarding the titrable acidity, it remained constant until the 6th day and then decreased slightly. It is known that the relation between the acidity and pH is not a simple inverse relationship because the phosphorous in the fruit acts a buffer. This is probably the reason why in some cases contradictory variations are noticed. For instance after 15 days at 25ºC the Redondo cultivar, presents higher acidity, however, a smaller pH value in comparison with day zero.

Differences in total soluble solids (TSS) depend on the cultivar variety, degree of maturation and agricultural techniques. The TSS values, measured by refractometry, are used as an index of total sugars in fruits, and indicate the degree of maturity. During storage, levels of TSS increased at temperatures of 12°C and 25°C, in three tomato cultivars (Redondo was the exception). Chucha cultivar presented the highest TSS increase at all tested temperatures, ranging from 2.9% when stored at 6ºC to 13.2% at 25ºC, followed by Cereja and Rama cultivars respectively. TSS increase observed during storage may be associated with the transformation of pectin substances, starch, hemi cellulose or other polysaccharides into soluble sugar (K. Singh & R. Singh, 2010). Redondo cultivar presented significant TSS decrease during storage, with a loss of 0.04%, 0.25% and 0.55% at 6ºC, 12ºC and 25ºC, respectively. According to Malumdo et al. (1995), total sugars differences may affect sensory attributes of tomatoes, particularly taste, sweetness and acidity. The storage time also interferes with sugars accumulation. Our results reveal that after the ninth storage day at 12 ºC all cultivars presented significant differences in the TSS ($p < 0.05$). Off all tomato cultivars analyzed, Cereja showed less pronounced differences with minor variations at 6ºC and increased variations in TSS (4.14 to 4.4 ºBrix), by the 15th day, when stored at 12ºC. Visual appearance is a critical factor driving the initial choice for purchase, but subsequent purchases are influenced greatly by eating satisfaction. The most important external characteristic to assess ripeness and postharvest life is tomato color (Messina et al., 2012). Initially all fruits presented a pink color. The effects of storage time and temperature on hue angle (ºh) for each tomato cultivar are shown in table 1. A decrease in hue values due to storage time was observed in all tomato cultivars studied; demonstrating that fruit color was darker and less yellow at day 15. Chucha variety exhibited significant differences in ºh values due to storage temperatures and Rama cultivar revealed a slight change in color index ($p > 0.05$). Color development in tomato is sensitive to temperature; a better plastid conversion is achieved when temperature is above 12ºC and below 30ºC (Gómez & Camelo, 2002; Dumas et al., 2003). On the other hand, at low temperature (below 12ºC), chlorophyll is not easily degraded and lycopene accumulation does not take place.

3.2 Bioactive Compounds and Antioxidant Capacity

Tomatoes are highly valued for their antioxidant content (Hanson et al., 2004; Rosales et al., 2006). Indeed, they possess as constituents several molecules with this capability of which we highlight lycopene, ascorbic acid and a variety of phenolic compounds (Rosales et al., 2011; Mohammed et al., 2012). The quantity of these molecules varies during the ripening process and with the cultivar (Valverde et al., 2011; Oms-Oliu et al., 2011). Our study sought to assess the role played by the conditions of postharvest storage (namely storage duration and temperature) in the contents of these molecules in four of the cultivars most consumed in Portugal. The results are presented in Table 2 and demonstrate difference in the content of these molecules between the different cultivars ($p < 0.05$).

Table 2. Evolution of the concentration of bioactive compounds present in the tomato fruits during storage at 6°C, 12°C and 25°C (± 1ºC) for 15 days. (AA) Ascorbic acid (mg/100g); (LC) Lycopene content (mg/100g); (TP) Total phenolic content (mg/100g)

Cultivar		First Day	3rd Day	6th Day	9th Day	12th Day	15th Day
Storage Temperature 6ºC							
Cereja	AA	72.05±1.02^{aA}	60.32±0.60^{bA}	71.11±1.12^{cA}	72.09±2.00^{cA}	72.09±2.25^{cA}	72.30±1.32^{cA}
	LC	60.1±1.73^{aA}	60.0±1.46^{aA}	59.7±0.09^{aA}	59.5±0.05^{aA}	56.1±1.23^{bA}	53.2±0.12^{cA}
	TP	40.95±1.28^{aA}	53.71±5.23^{bA}	66.15±3.34^{cA}	70.99±0.79^{dA}	55.81±1.73^{eA}	50.31±0.99^{fA}
Chucha	AA	39.77±0.93^{aA}	42.36±0.49^{bA}	49.62±1.00^{cA}	69.25±4.27^{dA}	59.00±1.07^{eA}	58.90±0.87^{eA}
	LC	50.9±1.74^{aA}	50.7±1.23^{aA}	48.7±0.09^{bA}	46.1±0.05^{bA}	43.1±0.06^{cA}	40.9±0.62^{dA}
	TP	86.81±1.44^{aA}	102.03±1.45^{bA}	105.57±2.98^{cA}	132.57±1.89^{dA}	86.32±0.78^{eA}	84.33±2.82^{eA}
Rama	AA	46.94±0.73^{aA}	45.44±0.48^{aA}	52.39±1.64^{bA}	55.12±1.02^{cA}	50.92±1.07^{cA}	52.13±4.15^{cA}
	LC	40.5±0.05^{aA}	40.5±0.02^{aA}	40.4±0.21^{aA}	40.5±0.08^{aA}	40.6±3.68^{aA}	40.5±4.96^{aA}
	TP	85.98±0.78^{aA}	133.69±0.31^{bA}	157.64±4.35^{cA}	172.17±2.24^{dA}	93.47±1.94^{eA}	91.20±1.14^{fA}
Redondo	AA	44.63±1.31^{aA}	26.68±3.38^{bA}	55.51±0.80^{cA}	57.87±0.76^{cA}	57.68±1.05^{cA}	57.46±1.55^{cA}
	LC	50.2±0.52^{aA}	50.7±0.87^{aA}	49.7±3.41^{aA}	48.6±2.55^{bA}	46.5±0.05^{bA}	43.9±3.47^{cA}
	TP	73.40±1.73^{aA}	109.68±1.16^{bA}	122.89±6.71^{cA}	121.65±2.90^{dA}	74.85±1.99^{eA}	72.22±1.42^{fA}
Storage Temperature 12ºC							
Cereja	AA	72.05±1.02^{aA}	59.97±0.78^{bA}	74.04±2.38^{cA}	76.56±0.50^{cB}	73.58±2.09^{dB}	70.22±0.84^{eB}
	LC	60.1±1.54^{aA}	60.1±0.05^{aA}	58.7±2.73^{bA}	55.2±0.84^{cB}	54.2±1.23^{cdB}	51.2±0.08^{deB}
	TP	40.95±1.28^{aA}	50.14±1.02^{bA}	64.22±2.77^{cA}	70.90±0.66^{dA}	70.99±1.08^{deA}	70.89±1.52^{deB}
Chucha	AA	39.77±0.93^{aA}	51.02±1.19^{bA}	55.89±1.18^{bA}	60.10±0.69^{cA}	56.12±2.74^{dA}	52.98±0.85^{eA}
	LC	50.9±0.99^{aA}	47.5±1.21^{bA}	41.2±3.64^{cB}	38.3±2.66c^{dB}	35.4±1.87^{dB}	32.9±0.34^{eB}
	TP	86.81±1.44^{aA}	94.10±4.47^{bA}	101.84±1.57^{cA}	115.63±2.32^{dB}	121.12±1.24^{eB}	117.96±2.50^{fB}
Rama	AA	46.94±0.73^{aA}	47.45±1.00^{aA}	55.75±0.96^{bA}	55.52±0.64^{bB}	51.71±0.83^{cB}	52.84±0.88^{cB}
	LC	40.5±1.45^{aA}	40.5±0.99^{aA}	40.2±0.86^{aA}	40.1±0.15^{aA}	40.1±1.27^{aB}	39.9±0.03^{bB}
	TP	85.98±0.78^{aA}	120.89±3.44^{bA}	152.29±2.08^{cA}	152.23±2.29^{cdB}	151.08±2.00^{eB}	147.54±1.84^{fB}
Redondo	AA	44.63±1.31^{aA}	36.23±0.75^{aA}	53.71±1.32^{bA}	60.77±0.76^{bA}	56.44±2.96^{cB}	50.46±0.88^{cB}
	LC	50.2±2.23^{aA}	49.9±0.78^{aA}	47.1±0.04^{bB}	44.9±0.09^{acA}	42.8±1.81^{dB}	42.01±0.71^{dB}
	TP	73.40±1.73^{aA}	104.65±2.89^{bA}	119.19±2.31^{cA}	112.86±1.97^{dB}	112.02±1.58^{eB}	102.49±1.69^{fB}
Storage Temperature 25ºC							
Cereja	AA	72.05±1.02^{aA}	71.41±0,83^{aA}	72.71±1.50^{aC}	68.65±3.39^{bC}	71.88±1.04^{abC}	72.08±0.68^{abC}
	LC	60.1±1.73^{aA}	55.6±1,46^{bB}	55.7±1.02^{bB}	51.8±2.15^{cC}	50.3±0.94^{dC}	48.8±0.92^{eC}
	TP	40.95±1.28^{aA}	29.18±1.12^{bB}	45.04±2.89^{cB}	41.22±1.17^{dB}	61.68±6.24^{eB}	41.67±1.63^{fB}
Chucha	AA	39.77±0.93^{aA}	60.98±0.87^{bB}	62.70±1.39^{bB}	58.91±1.11^{cB}	54.79±15.3^{cB}	21.03±0.19^{dB}
	LC	50.9±1.30^{aA}	39.4±1.16^{bB}	35.8±1.46^{cC}	34.2±0.87^{dC}	31.7±1.09^{eC}	30.2±1.46^{fC}
	TP	86.81±1.44^{aA}	64.47±3.67^{bB}	70.88±1.50^{cB}	75.08±3.34^{dC}	117.76±13.2^{eC}	90.91±1.35^{fC}
Rama	AA	46.94±0.73^{aA}	51.44±1.31^{aA}	52.08±1.37^{aB}	54.74±0.99^{bC}	49.83±5.77^{bC}	39.69±1.14^{cC}
	LC	40.4±1.80^{aA}	40.4±0.09^{aA}	42.0±2.00^{aB}	41.2±1.17^{aB}	40.5±1.12^{aC}	39.4±0.83^{aC}
	TP	85,98±0.78^{aA}	78.07±2.12^{bA}	83.63±2.90^{cB}	82.40±1.66^{dC}	157.96±25.47^{eC}	109.78±10.1^{fC}
Redondo	AA	44,63±1.31^{aA}	56.90±5.01^{bB}	57.69±2.18^{bB}	65.50±0.89^{cB}	54.76±7.12^{dC}	40.67±0.85^{eC}
	LC	50.2±2.50^{aA}	48.8±1.30^{aB}	43.2±3.70^{bC}	41.9±2.10^{cB}	40.5±0.79^{cdC}	40.8±0.67^{cdC}
	TP	73.40±1.73^{aA}	66.13±2.98^{bB}	71.05±0.92^{cB}	84.19±2.98^{dC}	119.76±9.65^{eC}	93.15±2.36^{fC}

*Values expressed as mean±standard deviation obtained from 6 measurements. For each cultivar, different lowercase superscripts indicate statistical significant differences caused by the storage time. Capital letters indicate significant differences related to different storage temperature.

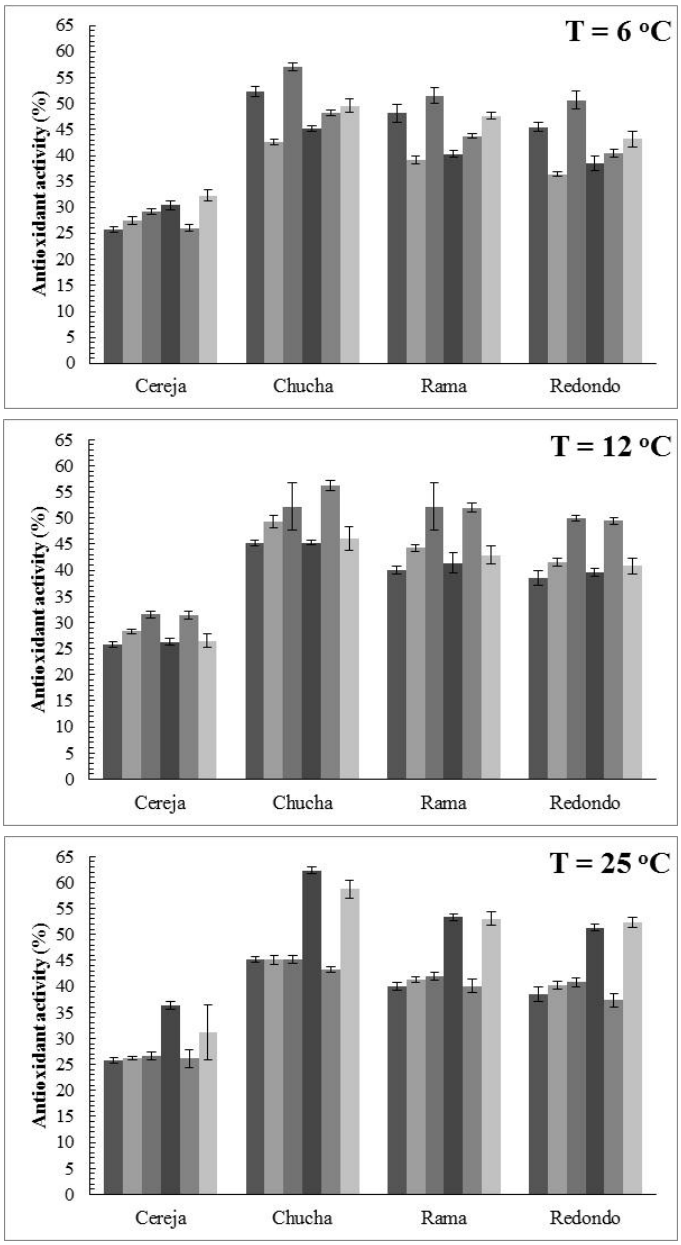

Figure 1. Antioxidant activity variation during storage time, for each Portuguese tomato cultivar (Cereja, Chucha, Rama e Redondo), at 6ºC, 12ºC and 25ºC. Each bar corresponds to a different day

Thus, it is observed that the concentration of ascorbic acid follows the sequence Chucha < Redondo < Rama < Cereja, the concentration of lycopene varies according to Redondo~Rama < Chucha~Cereja and that of phenolic compounds according to Cereja << Redondo < Rama~Chucha. The results also suggest that, in general, all molecules experience a positive change in their concentration with time, mainly for the tomatoes stored at 6°C and 12°C, reaching maximum concentration values around the ninth day. The phenolics decrease thereafter while the contents of the other molecules, with the exception of the tomatoes stored at 25°C, remain relatively stable. The decrease in levels of soluble phenolic compounds observed at the end of storage could be due to damage caused by reduced temperatures (6°C), particularly in the vacuoles of cells, the main compartment of phenolic compounds accumulation, and/or due to excess of rate of maturation fruits, when stored at higher temperatures (25ºC). Ascorbic acid registers only a slight accumulation during storage, in all conditions. It is recognized that high levels of acidity (see Table 1) are responsible for the stability of vitamin C during storage of fruits and vegetables. Furthermore, phenolic substances have also been linked to the stability of vitamin C due to its protective effect. Regarding the effect of the temperature, it is expressed mainly in the rate of change of concentrations rather than on their maximum values. For the same storage time it is observed that doubling the temperature from 6°C to 12°C

results in an increase in the levels of the various molecules. For example, Cereja tomatoes exhibited 1.5 times higher levels of lycopene at 12ºC, after 15 days, than at 6ºC. In Chucha and Redondo tomatoes, the increase was of 2 and 2.6 fold, respectively. In the previous section we reported that tomatoes stored at 12ºC and 25°C developed more color than the fruits stored at 6ºC. This is certainly linked with the increased concentrations of lycopene (negative significant correlations were observed). The influence of storage temperature on the level of lycopene has already been mentioned by other authors, who reported an increase in lycopene content between 3.6 and 9.0 mg/100g in tomatoes stored at 22°C for 14 days (Maul et al., 2000). The effect of temperature is not as clear when it is increased to 25°C. In fact the opposite is observed regarding the total phenolic content. On the same day of observation, the specimens kept at 25°C present lower phenolic compound levels than those kept at 12°C. Moreover, we observed that the tomatoes stored at 25°C showed, in general, deterioration in their values of bioactive compounds from the 12th day of storage. Since antioxidant activity is related to the contents of lycopene, ascorbic acid and phenolic compounds (positive correlations were observed in all cases) it is not surprising that it follows a trend that is parallel with those observed for those molecules (Figure 1). After 15 days of storage, a higher antioxidant activity was observed in comparison to the start of the experiment, with values ranging on average between 2% and 13.8%. These data are consistent with other studies (Dumas et al., 2003; Toor & Savage, 2006) which attributed 92% of the antioxidant activity to hydrophilic compounds and only 8%, on average, for the lipophilic fraction.

3.3 Microbial Contamination

Microbial contamination of tomatoes can be a serious cause of their deterioration and represents a health hazard to the consumer. The most common microorganisms found in postharvested tomatoes are mesophyll, coliforms, molds and yeast. Temperature is one of the environmental factors that most affects microbial growth and activity, due its influence on enzymatic activity of tissues and microbes. The results of the microbiological tests performed on the cultivars studied are presented in Table 3.

Table 3. Determination of colony forming units at day zero and 15 days after storage at different temperatures.

	Cultivar	Mesophyll*			Coliforms*			Molds*		
Day 0	Cereja	-			-			-		
	Chucha	++			++			+		
	Rama	+			-			-		
	Redondo	+			+			+		
		6ºC	**12ºC**	**25ºC**	**6ºC**	**12ºC**	**25ºC**	**6ºC**	**12ºC**	**25ºC**
Day 15	Cereja	-	-	-	-	-	-	-	-	-
	Chucha	+	+	++	+	++	+++	+	+	++
	Rama	-	-	+	-	-	+	-	+	+
	Redondo	-	+	++	+	+	+++	-	+	++

* (-) CFU/g<10^2; (+) [10^2≤CFU/g<10^4]; (++) [10^4≤CFU/g<10^5]; (+++) [10^5≤CFU/g≤10^8]. The values presented are the means of 6 measurements.

At day 0 the mesophyll count was found to be >10^2 CFU/g in three cultivars, Rama (1.0 x 10^2 CFU/g), Redondo (2.7 x 10^3 CFU/g) and Chucha (4.9 x 10^4 CFU/g). These levels of contamination may derive from the direct contact with soil, irrigation water and/or contact with the handler, among others. The bigger fruits (Chucha and Redondo), germinate and grow at the bottom of the plant, promoting direct contact with soil, while Cereja tomatoes, much smaller, are suspended at the top of the plant. After the storage period, mesophyll counts increased drastically in cv. Chucha for all tested temperatures (1.9 x 10^3, 1.4 x 10^3, 8.3 x 10^5), in cv. Redondo, counts increased at 12ºC and 25ºC (1.7 x 10^2 and 7.1 x 10^5, respectively). cv. Rama was among the three cultivars initially infected, the one who presented lower counts (1.9 x 10^2 CFU/g) at 25°C. These results can be partially explained taking into account the physiological profile of each cultivar, as the average moisture content for cv. Chucha is 90%, while cv. Rama has only 84% (higher moisture content is more favorable to microbial contamination). Regarding the total coliforms, the counts revealed significant contamination present only in the cultivars Chucha and Redondo, this can be related to the, already mentioned, fact that these fruits grow in direct contact with the soil.

Results reveal that storage temperature strongly affects total coliforms growth. Temperature is determinant in the inhibition and delaying of the activity of microbial enzymes and non-enzymatic chemical reactions. Storage at 6ºC and 12ºC inhibited coliform bacterial growth. At 25ºC the Chucha contamination, initially at 2.2 x 10^5 increased exponentially to 1.8 x 10^8 CFU/g. The Redondo cultivar was the one that presented the highest level of total coliforms growth: the initial 2.2 x 10^3 CFU/g resulted, after 15 days at 25ºC, in 5.7 x 10^8 CFU/g. *Escherichia coli* was not detected in any tomato cultivar studied throughout the experiment (data not shown). Grey molds and yeast were counted separately. None of the cultivars studied exhibited or developed yeast contaminations throughout the experiment. At day zero, there were counts of 1.2 x 10^2 molds on Chucha and 1.4 x 10^2 for Redondo. Cultivars Cereja and Rama did not present molds. During storage it was observed a severe loss of firmness on Chucha cultivar specimens, presenting more contamination on the 15th day. Colonies amounted to 4.6 x 10^2, 1.9 x 10^3 and 3.6 x 10^5 CFU/g in Chucha tomatoes, at 6, 12 and 25°C, respectively. The colony counts recorded for Redondo tomato cultivar, at the same temperature were 2.2 x 10^1, 1.8 x 10^2 and 8.0 x 10^4 CFU/g, while the mold count in Rama tomato was only positive at 12°C and 25°C with 1.5 x 10^2 and 1.6 x 10^2 CFU/g, respectively.

Microbiological characterization of tomato is highly important as this fruit is consumed fresh. The main causes of contamination are related to hygiene, inadequate farming equipment, the latter may become sources of contamination, especially by molds and yeasts, also bolstering cross-contamination, which source can be the raw material, air, dust and even handler agriculture (Ayala-Zavala et al., 2008). Coliforms are organisms related to undesirable or precarious sanitary conditions. The presence of these microorganisms does not necessarily indicate fecal contamination, as the majority of coliforms are found in the environment. Contaminations of this kind are confirmed only by the presence of *Escherichia coli* (Kaper et al., 2004; Gyles & Fairbrother, 2010). The consumer must be aware that good product appearance is not a synonym of absence of microbial contamination. Cereja was the only cultivar studied that was found free of any sort of microorganism contamination, during all the experiment.

The analysis of the microbiological parameters during storage points towards the existence of a correlation with physiological parameters of each tomato cultivar. Chucha tomato reveals increased contamination and growth of microbial flora during storage, a result that can be related to its high pH (pH = 4.5) and high moisture content (90%).

4. Conclusions

The present study demonstrates the effect of storage time and temperature on physiological, biochemical and microbiological parameters of four Portuguese tomato cultivars namely, Cereja, Chucha, Rama and Redondo. Globally, the effect of storage time was more pronounced ($p < 0.01$) than that of the storage temperature (mean values with a statistical significance of ($p < 0.05$). Physiological parameters were the most affected during storage period ($p < 0.01$). The antioxidant compounds; lycopene, ascorbic acid and total phenolic contents increased over time in all analyzed cultivars. Even though storage time promoted the synthesis of lycopene, storage temperature was extremely relevant in color variation. The microbial flora was observed showing top scores in cultivars growing in small bushes and at the bottom of its stem, increasing the contact surface contaminants.

This study reveals that the cultivars studied have a positive development on nutritional content up to a maximum of nine days storage and the ideal temperature to maintain optimal physiological, biochemical and microbiological profiles is 12ºC.

In spite of the similar antioxidant activity between cv. Rama and cv. Redondo, Rama fruits. offer greater resistance to microbial contamination ($p < 0.05$), at the same storage conditions, allowing the conclusion that Rama is the fruit most suitable for fresh use, where thermal conditions of hygienic handling and storage may be very variable, minimizing the risk for public health.

References

Anthon, G. E., LeStrange, M., & Barrett, D. M. (2011). Changes in pH, acids, sugars and other quality parameters during extended vine holding of ripe processing tomatoes. *J. Sci. Food Agric., 91*, 1175-1181. http://dx.doi.org/10.1002/jsfa.4312

AOAC International. (2005). *Official Methods of Analysis*. Maryland, USA: AOAC International.

Arias, R., Lee, T. C., Logendra, L., & Janes, H. (2000). Correlation of lycopene measured by HPLC with the *L**, *a**, *b** color readings of a hydroponic tomato and the relationship of maturity with color and lycopene content. *J. Agric. Food Chem., 48*, 1697-1702. http://dx.doi.org/10.1021/jf990974e

Ayala-Zavala, J. F., Del-Toro-Sánchez, L., Alvarez-Parrilla, E., & González-Aguilar, G. A. (2008). High Relative Humidity In-Package of Fresh-Cut Fruits and Vegetables: Advantage or Disadvantage Considering

Microbiological Problems and Antimicrobial Delivering Systems? *J. Food Sci., 73*(4), 41-47. http://dx.doi.org/10.1111/j.1750-3841.2008.00705.x

Babatola, L. A., Ojo, D. O., & Lawal, O. I. (2008). Effect of storage conditions on tomato (*Lycopersicon esculentum* Mill.). Quality and shelf life. *J. Biol. Sci., 8*(2), 490-493. http://dx.doi.org/10.3923/jbs.2008.490.493

Batu, A. (2004). Determination of acceptable firmness and colour values of tomatoes. *J. Food Eng.*, *61*(3), 471-475. http://dx.doi.org/10.1016/S0260-8774(03)00141-9

Carlsen, M. H., Halvorsen, B. L., Holte, K., Bohn, S. K., Dragland, S., Sampson, L., … Blomhoff, R. (2010). The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. *Nutr. J., 9*(3), 2-11. Retrieved from http://www.nutritionj.com/content/9/1/3

Choudhary, R., Bowser, T. J., Weckler, P., Maness, N. O., & McGlynn, W. (2009). Rapid estimation of lycopene concentration in watermelon and tomato puree by fiber optic visible reflectance spectroscopy. *Postharvest Biol. Technol., 52*, 103-109. http://dx.doi.org/10.1016/j.postharvbio.2008.10.002

Dumas, Y., Dadomo, M., Lucca, G., & Grolier, P. (2003). Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. *J. Sci. Food Agric., 83*, 369-382. http://dx.doi.org/10.1002/jsfa.1370

Gómez, P. A., & Camelo, A. F. L. (2002). Calidade postcosecha de tomates almacenados em atmósferas controladas. *Hortic Bras, 20*(1), 38-43. http://dx.doi.org/10.1590/S0102-05362002000100007

Gyles, C. L., & Fairbrothe, R. J. M. (2010). Escherichia coli. In C. L. Gyles, J. F. Prescott, J. G. Songer, C. O. Thoen, I. A. Ames, (Eds.), *Pathogenesis of Bacterial Infections in Animals* (pp. 267-308). Wiley-Blackwell.

Hanson, P. M., Yang, R. Y., Wu, J., Chen, J. T., Ledesma, D., & Tsou, S. C. S. (2004). Variation for antioxidant activity and antioxidants in tomato. *J. Amer. Soc. Hort. Sci., 129*(5), 704-711. Retrieved from http://journal.ashspublications.org/content/129/5/704

Jang, H. D., Chang, K. S., Huang, Y. S., Hsu, C. L., Lee, S. H., & Su, M. S. (2007). Principal phenolic phytochemicals and antioxidant activities of three Chinese medicinal plants. *Food Chem., 103*, 749-756. http://dx.doi.org/10.1016/j.foodchem.2006.09.026

Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004). Pathogenic *Escherichia coli*. *Nat Ver Microbiol, 2*, 123-140. http://dx.doi.org/10.1038/nrmicro818

Korekar, G., Stobdan, T., Singh, H., Chaurasia, O. P., & Singh, S. B. (2011). Phenolic content and antioxidant capacity of various solvent extracts from Seabuckthorn (*Hippophae rhamnoides* L.) fruit pulp, seeds, leaves and stem bark. *Acta Aliment, 40*(4), 449-458. http://dx.doi.org/10.1556/AAlim.40.2011.4.4

Malumdo, T. M. T., Shewfelt, R. T., & Scott, J. W. (1995). Flavor quality of fresh tomato (*Lycopersicon esculentum* Mill.) as affected by sugar and acid levels. *Postharvest Biol Technol, 6*, 103-110. http://dx.doi.org/10.1016/0925-5214(94)00052-T

Maul, E., Sargent, S. A. Sims, C. A. Baldwin, E. A., Balaban, M. O., & Huber, D. J. (2000). Tomato flavor and aroma quality as affected by storage temperature. *J. Food Sci.*, *65*(7), 1228-1237. http://dx.doi.org/jfsv65n7p1228-1237ms19990912

Messina, V., Domínguez, P. G., Sancho, A. M., Reca, N. W., Carrari, F., & Grigioni, G. (2012). Tomato quality during short-term storage assessed by colour and electronic nose. *Int. J. Electrochem, 2012*, 1-7. http://dx.doi.org/10.1155/2012/687429

Mohammed, A. E., Smit, I., Pawelzik, E., Keutgen, A. J., & Horneburg, B. (2012). Organically grown tomato (*Lycopersicon esculentum* Mill.): bioactive compounds in the fruit and infection with *Phytophthora infestans*. *J. Sci. Food Agric., 92*, 1424-1431. http://dx.doi.org/10.1002/jsfa.4720.

Moneruzzaman, K. M., Hossain, A. B. M. S., Sani, W., Saifuddin, M., & Alenazi, M. (2009). Effect of harvesting and storage conditions on the post harvest quality of tomato (*Lycopersicon esculentum* Mill) cv. Roma VF. *Aust. J, Crop Sci., 3*(2), 113-121. Retrieved from http://www.researchgate.net/.../26591563

NP 3277 - Microbiologia Alimentar. (1987). Contagem de bolores e leveduras, parte 1- incubação a 25ºC.

NP 3788- Microbiologia Alimentar. (1990). Regras gerais para a contagem de bactérias coliformes a 30ºC.

NP 4396 - Microbiologia Alimentar. (2002). Regras gerais para contagem de *Escherichia coli*.

NP 4405 - Microbiologia Alimentar. (2002). Regras gerais para contagem de microorganismos; contagem de colónias a 30ºC.

Okolie, N. P., & Sanni, T. E. (2012). Effect of post harvest treatments on quality of whole tomatoes. *Afric. J. Food Sci.*, *6*(3), 70-76. http://dx.doi.org/10.5897/AJFS11.188

Oms-Oliu, G., Hertog, M. L. A. T. M., Van de Poel, B., Ampofo-Asiama, J., Geeraerd, A. H., & Nicolaï, B. M. (2011). Metabolic characterization of tomato fruit during preharvest development, ripening, and postharvest shelf-life. *Postharvest Biol Technol, 62*, 7-16. http://dx.doi.org/10.1016/j.postharvbio.2011.04.010

Pantheen, D. R., & Chen, F. (2010). Genomics of fungal disease resistance in tomato. *Curr Genomics, 11*(1), 30-39. http://dx.doi.org/10.2174/138920210790217927

Požrl, T., Žnidarčič, D., Kopjar, M., Hribar, J., & Simčič, M. (2010). Change of textural properties of tomatoes due to storage and storage temperatures. *J. Food Agric. & Envir., 8*(2), 292-296. Retrieved from www.isfae.org/scientficjournal/2010/issue2/pdf/food/f58.pdf

Reis, E. R., Finger, F. L., Valle, R. H. P., & Nery, F. C. (2003). Avaliação da qualidade microbiológica de mini-milho (Zea Mays) minimamente processado. *Rev. Hig. Alim.*, *17*(110), 66-68. Retrieved from http://bases.bireme.br/cgi/

Rosales, M. A., Cervilla, L. M., Sánchez-Rodríguez, E., Rubio-Wilhelmi, M. M., Blasco, B., Ríos, J. J., ... Ruiz, J. M. (2011). The effect of environmental conditions on nutritional quality of cherry tomato fruits: evaluation of two experimental Mediterranean greenhouses. *J. Sci. Food Agric., 91*, 152-162. http://dx.doi.org/ 10.1002/jsfa.4166.

Rosales, M. A., Ruiz, J. M., Hernandez, J., Soriano, T., Castilla, N., & Romero, L. (2006). Antioxidant content and ascorbate metabolism in cherry tomato exocarp in relation to temperature and solar radiation. *J. Sci. Food Agric., 86*, 1545-1551. http://dx.doi.org/10.1002/jsfa.2546

Sadler, G., Davis, J., & Dezman, D. (1990). Rapid extraction of lycopene and β-carotene from reconstituted tomato paste and pink grapefruits homogenates. *J Food Sci, 55*, 1460-1461. http://dx.doi.org/10.1111/j.1365-2621.1990.tb03958.x

Saeed, S., Rashid, N., Tahir, A., & Jones, P. G. (2009). 1-(4-Bromophenyl)-1-(4-nitrobenzoyl)-thiourea. *Acta Cryst, E65*, 1870-1871. http://dx.doi.org/10.1107/S1600536809025884

Sánchez-Moreno, C., Plaza, L., De Ancos, B., & Cano, M. P. (2006). Nutritional characterization of commercial traditional pasteurized tomato juices: carotenoids, vitamin C and radical-scavenging capacity. *Food Chem., 98*, 749-756. http://dx.doi.org/ 10.1016./j.foodchem.2005.07.015.

Singh, R. K., & Singh, R. N. (2010). Effect of post harvest treatments on self life of mango (*Mangifera indica* L.) fruits cv. Amrapali. *Research J. Agric. Sci., 1*(4), 415-418. Retrieved from http://www.rjas.info

Tang, J., & Wang, T. (2003). Dehydrated vegetables: Principles and System. In Y. H. Hui, S. Ghazala, D. M. Graham, K. D. Murrell, & W. K. Nip (Eds.), *Handbook of Vegetable Preservation and Processing* (pp. 335-372). New York, USA: CRC Press. Retrieved from http://www.doi.org/1201/9780203912911.fmatt

Toor, R. K., & Savage, G. P. (2006). Effects of semi-drying on the antioxidant components of tomatoes. *Food Chem, 94*, 90-97. http://dx.doi.org/ 10.1016./j.foodchem.2004.10.054

Valverde, V. G., González, I. N., Alonso, J. G., & Periago, M. J. (2011). Antioxidant Bioactive Compounds in Selected Industrial Processing and Fresh Consumption Tomato Cultivars. *Food Bioprocess Technol*, 687-369. http://dx.doi.org/ 10.1007/s11947-011-0687-3

Vinha, A. F., Soares, M. O., Herdeiro, M., & Machado, M. (2012). Chemical composition and antioxidant activity of Portuguese diospyrus kaki fruit by geographical origins. *J. Agric. Sci., 4*(2), 281-289. http://dx.doi.org/10.5539/jas.v4n2p281

Zieliski, H., & Kozowska, H. (2000). Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. *J. Agric. Food Chem., 48*, 2008-2016. http://dx.doi.org/10.1021/jf990619o