

**Physical, physicochemical, microbiological, and bioactive compounds stability of low-calorie orange jellies during storage: packaging effect**

**Estabilidade física, físico-química, microbiológica e dos compostos bioativos de geleias de laranja de baixa valor calórico durante o armazenamento: efeito das embalagens**

**Estabilidad física, físico-química, microbiológica y compuestos bioactivos de las jaleas de naranja bajas en calorías durante el almacenamiento: efecto de envasado**

Received: 26/08/2020 | Reviewed: 02/09/2020 | Accept: 05/09/2020 | Published: 06/09/2020

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### **Abstract**

Fruit jellies are widely produced as a way to utilize fresh fruits, which are highly perishable. Orange a fruit widely consumed in Brazil, it has a significant amount of bioactive compounds. Despite the great progress in the development of jellies, several factors can change its useful life, among them is the packaging. Therefore, the objective of this study was to evaluate the effect of packaging on the physicochemical, physical, microbiological and bioactive compounds stability of low-calorie orange jellies during storage. Analyses every 30 days during the 180 days of storage. The results showed that increased storage time led to a decrease in pH, reduction of the flow rate (polypropylene packaging), reduction in yellow intensity, and growth of fungi and yeasts (higher in polypropylene packaging). In contrast, luminosity, red intensity, moisture, total sugars, and the consistency index tended to remain stable during storage. The DPPH results showed an increase in the antioxidant activity and reduction of vitamin C throughout the period of storage, especially in polypropylene packaging. The total phenolic content was stable with a tendency to decrease during storage. Notably, vitamin C showed a positive correlation with antioxidant activity in jellies. Low-calorie orange jellies packaged in glass showed the least changes during storage.

**Keywords:** Antioxidant activity; Food quality; Rheology; Shelf life; Stability.

### **Resumo**

As geleias de frutas são amplamente produzidas como forma de aproveitar as frutas frescas, que são altamente perecíveis. A laranja é uma fruta amplamente consumida no Brasil, possui uma quantidade significativa de compostos bioativos. Apesar do grande progresso no desenvolvimento de geleias, vários fatores podem alterar sua vida útil, entre eles está a embalagem. Portanto, o objetivo deste estudo foi avaliar o efeito das embalagens na estabilidade físico-química, física, microbiológica e dos compostos bioativos de geleias de

laranja de baixo valor calórico durante o armazenamento. Foram realizadas análises a cada 30 dias durante os 180 dias de armazenamento. Os resultados mostraram que o aumento do tempo de armazenamento levou à diminuição do pH, redução índice de fluxo (embalagem de polipropileno), redução da intensidade do amarelo e crescimento de fungos e leveduras (maior na embalagem de polipropileno). Em contraste, a luminosidade, a intensidade do vermelho, a umidade, os açúcares totais e o índice de consistência tenderam a permanecer estáveis durante o armazenamento. Os resultados do DPPH mostraram aumento da atividade antioxidante e redução da vitamina C ao longo do período de armazenamento, principalmente nas embalagens de polipropileno. O teor dos compostos fenólicos totais manteve-se estável com tendência a diminuir durante o armazenamento. Notavelmente, a vitamina C mostrou uma correlação positiva com a atividade antioxidante em geleias. Geleias de laranja de baixa caloria embaladas em vidro mostraram as menores alterações durante o armazenamento.

**Palavras-chave:** Atividade antioxidante; Qualidade alimentar; Reologia; Vida útil; Estabilidade.

### Resumen

Las jaleas de frutas se producen ampliamente como una forma de aprovechar las frutas frescas, que son altamente perecederas. La naranja es una fruta muy consumida en Brasil, tiene una cantidad significativa de compuestos bioactivos. A pesar del gran avance en el desarrollo de las jaleas, varios factores pueden cambiar su vida útil, entre ellos se encuentra el empaque. Por tanto, el objetivo de este estudio fue evaluar el efecto de los envases sobre la estabilidad físico-química, física, microbiológica y los compuestos bioactivos de las jaleas de naranja bajas en calorías durante el almacenamiento. Análisis a cada 30 días durante 180 días de almacenamiento. Los resultados mostraron que el aumento del tiempo de almacenamiento condujo a una disminución del pH, reducción del índice de flujo (empaque de polipropileno), reducción de la intensidad del amarillo y crecimiento de hongos y levaduras (mayor en el empaque de polipropileno). Por el contrario, la luminosidad, la intensidad del rojo, la humedad, los azúcares totales y el índice de consistencia tendieron a permanecer estables durante el almacenamiento. Los resultados de DPPH mostraron un aumento de la actividad antioxidante y una reducción de la vitamina C durante el período de almacenamiento, especialmente en los envases de polipropileno. El contenido de compuestos fenólicos totales se mantuvo estable con tendencia a disminuir durante el almacenamiento. En particular, la vitamina C mostró una correlación positiva con la actividad antioxidante en jaleas. Las

gelatinas de naranja bajas en calorías envueltas en vidrio mostraron los cambios más pequeños durante el almacenamiento.

**Palabras clave:** Actividad antioxidante; Calidad de la comida; Reología; Vida útil; Estabilidad.

## 1. Introduction

Consumption of fruits and fruit products is of paramount importance due to their various health benefits. Fruits have anti-inflammatory and antioxidant properties, which contribute to individual health. In addition, they can promote low-energy intake, because they contain fiber, which increases satiety (Souza et al., 2014). The orange (*Citrus sinensis* L. Osbeck) of the family Rutaceae has a high content of bioactive compounds represented mainly by vitamin C. Vitamin C favors the elimination of free radicals, helps protect cells against oxidative damage and flavonoids that have anti-inflammatory and anticancer activities, improves blood pressure and lipid profile, and reduces risk of cardiovascular diseases (Coelho et al., 2013).

Fruit jellies are innovative, practical, and functional products that can be used to preserve fruits, which are highly perishable (Souza et al., 2014). However, because of the increase in chronic lifestyle-related diseases, such as diabetes and obesity, there is a growing need for low-calorie products, such as low-calorie jellies (Abolila et al., 2015). To obtain low-calorie jellies, it is necessary to reduce the amount of sugar. However, it is also necessary to add sweeteners, to impart a sweet flavor; body agents, to give form to the product; and gelling agents, to aid in their formation (Nachtigall et al., 2004). It is important to verify the stability of these ingredients during storage, as their stability will directly affect the shelf life of final product (Santos et al., 2019).

Despite much progress, food storage stability is still a complex issue due to the numerous factors that can reduce shelf life. In fruit jellies, changes in acidity, sugar hydrolysis, increased or decreased moisture, darkening, increased or decreased consistency, syneresis, and the growth of microorganisms, mainly mold and yeasts, can cause product deterioration (Hayat et al., 2005; Javanmard & Endan, 2010). The bioactive compounds are very unstable and can be degraded during processing and storage (Igual et al., 2015). The packaging in which the food is confined may exert an influence on the stability of bioactive compounds, especially the permeability of the container to oxygen, which favors the degradation of these compounds, or the presence of a barrier to the entrance of light (Pérez-

Vicente et al., 2004). It is important that the food industry is aware about the degradation of these bioactive compounds since this factor influences the shelf life of foods, especially those that have a functional food claim (Shimoni 2004; Zulueta et al., 2010).

Therefore, the objective of this study was to evaluate the effect of packaging on the physicochemical, physical, microbiological and bioactive compounds stability of low-calorie orange jellies during storage.

## **2. Materials and Methods**

### **2.1 Materials**

The following materials were used: oranges (Pêra Rio cultivar), crystal sugar (Alvinho, Governador Valadares, Brazil), polydextrose (Nutramax, Catanduva, Brazil), carrageenan gum (Gastronomy Lab, Distrito Federal, Brazil), low methoxylated pectin (Rica Nata, Piracema, Brazil), sucralose, acesulfame-k (Nutramax, Catanduva, Brazil), and potassium sorbate (Rica Nata, Piracema, Brazil).

### **2.2 Processing of oranges and jellies**

After washing the oranges, the fruit was soaked in a 2.5% sodium hypochlorite solution for 15 minutes to sanitize them and avoid contamination of the fruit pulp. Then, orange juice was obtained by processing the fruits in an electric juicer without adding sugar or water. The juice was stored at -18 °C in polyethylene pots covered with aluminum foil.

The jellies were prepared in an open pan heated by a gas flame as follows according to the method of Lima et al. (2019). First, orange juice (60%), crystal sugar (20%), and polydextrose (18.925%) were mixed. Then, the mixture was heated at 80 °C to 30° Brix, and then the gelling agents, low methoxylated pectin (0.7%) and gum carrageenan (0.3%) dissolved in 5 mL of distilled water, were added. Next, the mixture was baked to 60° Brix. Then, the sweeteners acesulfame-k and sucralose were added, as described by Souza et al. (2013), as well as the preservative potassium sorbate (0.05%) dissolved in 2 mL of distilled water. The jellies were cooked until a final soluble solid with a value of 65° Brix was achieved. Then, the jellies were hot packed in previously sterilized transparent glass and polypropylene (PP) jars (glass jars were sterilized in boiling water for 10 min. and polypropylene jars were sterilized in water with 2.5% sodium hypochlorite for 20 min.) and

stored in an incubator chamber at 25 °C for 6 months.

### **2.3 Physicochemical evaluation of low-calorie orange jellies**

The moisture, acidity, and pH were analyzed according to IAL (2008) and AOAC (2011). The method of Dische (1962) was used to determine total sugars. The analyses were performed in triplicate.

### **2.4 Physical evaluation of low-calorie orange jellies**

The color of the jellies was determined using a Konica Minolta colorimeter, model CR 400, working in D65 (daylight) and using CIELab standards, according to the method of Lau et al. (2000) in quadruplicate.

The rheological analysis were performed in a cone/plate-type rheometer (Brookfield model RV-III) using Rheocalc software (version 3.0), spindle CP52, and 0.5 g of sample. To obtain the upward curve, the speed of rotation was 1–250 rpm, which was increased in 5 intervals at 50 rpm. To obtain the downward curve, the procedure was repeated in the reverse direction, with progressively decreasing velocities (250–1 rpm). The measurements were performed in triplicate.

Syneresis was evaluated according to the method of Licodiedoff et al. (2010).

### **2.5 Microbiological evaluation**

The microbiological quality of the jellies was evaluated by determining the numbers of molds and yeasts that were determined by plating the homogenate on PDA (Potato Dextrose Agar), acidified with tartaric acid 10%. The results were expressed in colony forming units per gram of jelly (CFU/g) and compared with the standards established by DRC n° 12 (Brazil, 2001).

### **2.6 Bioactive compounds evaluation**

The stability of bioactive compounds was assessed by means of determination of ascorbic acid, total phenolic content and antioxidant activity (ABTS, DPPH and  $\beta$ -carotene methods).

### **2.6.1 Determination of ascorbic acid (Vitamin C)**

For the determination of the ascorbic acid content, standard AOAC (1984) methodology modified by Benassi & Antunes (1988) was used. Dilution of the samples was done in 100 mL of 2% oxalic acid solution, and a 25 mL aliquot was then titrated with 0.025% DCFI (2,6-dichlorophenolindophenol) solution until pink coloration was obtained. The solution was previously standardized with L-ascorbic acid solution. The results are expressed as mg ascorbic acid/100 g of fw.

### **2.6.2 Obtaining extracts of samples for analysis of phenolic content and antioxidant activity**

The extracts were obtained according to the method described in a previous study (Larrauri et al., 1997). Ten grams of the sample was weighed, followed by the addition of 40 mL of methanol/water solution (50:50 v/v). This mixture was kept under stirring (200 rpm) at room temperature for 60 min and then allowed to rest in a cooled (8 °C) environment for 30 min. The supernatant was then recovered, filtered, and transferred to a 100 mL flask. Next, Forty millilitres of acetone/water (70:30, v/v) was added to the residue, maintaining stirring (200 rpm) at room temperature for 60 min, and then allowed to stand in a cooled (8 °C) environment for 30 min. The methanol and acetone extracts were combined and brought to a final volume of 50 mL with distilled water for the determination of antioxidant activity, total phenolic content.

### **2.6.3 Total phenolic content**

The total phenolic content was determined according to the adapted Folin–Ciocalteu method (Waterhouse, 2002). The extracts (0.5 mL) were mixed with 2.5 mL of Folin–Ciocalteu reagent (10%) and 2 mL of sodium carbonate solution (4%). The mixture was stirred and kept at room temperature for 2 h in the dark. The absorbance was measured at 750 nm against a blank. Aqueous solutions of gallic acid were used for calibration (10-80 µg mL<sup>-1</sup>). The results are expressed as gallic acid equivalents (GAE)/g of orange jelly.

#### 2.6.4 Antioxidant activity

The antioxidant activity was determined using the ABTS, DPPH and  $\beta$ -carotene methods. For the ABTS assay, the procedure followed the previous method described by Re et al. (1999) with few modifications. The 2,2-azinobis (ABTS) radical cation (ABTS $\bullet$ +) was generated by reaction of 5 mL of aqueous ABTS solution (7 mM) with 88  $\mu$ L of 140 mM (2.45 mM final concentration) potassium persulphate. The mixture was kept in the dark for 16 h before use and then diluted with ethanol to obtain an absorbance of  $0.7 \pm 0.05$  units at 734 nm using a spectrophotometer VIS 325–1000 nm. The jelly extracts (30  $\mu$ L) or a reference substance (Trolox) was put to react with 3 mL of the resulting blue-green ABTS radical solution in the dark. The decrease of absorbance at 734 nm was measured after 6 min. Ethanolic solutions of known Trolox concentrations were used for calibration (100–2.000  $\mu$ M). The results are expressed as micromoles of Trolox equivalents (TEs) per gram of jelly ( $\mu$ mol of TEs g<sup>-1</sup> of jelly).

DPPH-free radical scavenging capacity was estimated using the method reported by Brand-Williams et al. (1995). Briefly, the DPPH solution (600  $\mu$ M) was diluted with ethanol to obtain an absorbance of  $0.7 \pm 0.02$  units at 517 nm. Jelly extracts (0.1 mL) were put to react with 3.9 mL of DPPH radical solution for 120 min in the dark, and the decrease in absorbance of the resulting solution was monitored. The absorbance of the reaction mixture was measured at 517 nm. The results were expressed as EC<sub>50</sub> (g of jelly/g of DPPH).

The antioxidant activity was also determined by the  $\beta$ -carotene method, following the procedure described by Marco (1968) with minor modifications. Briefly, an aliquot (50  $\mu$ L) of the  $\beta$ -carotene chloroform solution (20 mg/mL) was added to a flask containing 40  $\mu$ L of linoleic acid, 1.0 mL of chloroform, and 530  $\mu$ L of Tween 40 and then mixed. The chloroform was evaporated using an oxygenator. After the evaporation, oxygenated distilled water (approximately 100 mL) was added to obtain an absorbance of  $0.65 \pm 0.5$  units at 470 nm. An aliquot (0.4 mL) of Trolox solution (200 mg/L) or diluted jelly extract (200 mg/L) was added to 5 mL of the  $\beta$ -carotene solution and incubated in a water bath at 40 °C. The measurements were performed after 2 min and 120 min at an absorbance of 470 nm using a spectrophotometer. The antioxidant activity was calculated as the percent inhibition relative to the control.



## **2.7 Experimental design and statistical analysis**

The experimental design was a complete 2×7 factorial, and the factors under study were packaging (glass and polypropylene-PP) and storage time (0, 30, 60, 90, 120, 150, and 180 days).

The data were evaluated by analysis of variance (ANOVA), Tukey's test, and regression at 5% significance using Sisvar software (Ferreira, 2014). The antioxidant activity was calculated according to the equation proposed by Duarte-Almeida et al. (2006). Correlation was performed using the means of the antioxidant activity by the DPPH method and the means of the results for vitamin C and phenolic content, which was calculated in Excel 2013 using the CORREL function. The standard error of the mean (SEM) was determined by calculating the standard deviation of the sample divided by the square root of the total number of values obtained in the analysis during the 180 days, for each type of packaging.

## **3. Results and Discussion**

### **3.1 Physicochemical evaluation of low-calorie orange jellies**

Table 1 presents the average physicochemical parameters of the low-calorie orange jellies over 180 days of storage in glass and PP packaging.

**Table 1.** Evaluation of the physicochemical parameters of low-calorie orange jellies in relation to the packaging (polypropylene and glass) and storage time (180 days).

Variables	Packaging	TIME (days)						
		0	30	60	90	120	150	180
pH	PP	4,44 ± 0,02 <sup>Aa</sup>	4,27 ± 0,02 <sup>Ac</sup>	4,19 ± 0,02 <sup>Bd</sup>	4,34 ± 0,02 <sup>Ab</sup>	4,27 ± 0,03 <sup>Ac</sup>	4,34 ± 0,01 <sup>Bb</sup>	4,28 ± 0,01 <sup>Ac</sup>
		4,44 ± 0,02 <sup>Aa</sup>	4,27 ± 0,01 <sup>Ad</sup>	4,22 ± 0,02 <sup>Ae</sup>	4,34 ± 0,02 <sup>Ac</sup>	4,28 ± 0,02 <sup>Ad</sup>	4,36 ± 0,01 <sup>Ab</sup>	4,27 ± 0,01 <sup>Ad</sup>
	Glass	4,44 ± 0,02 <sup>Aa</sup>	4,27 ± 0,01 <sup>Ad</sup>	4,22 ± 0,02 <sup>Ae</sup>	4,34 ± 0,02 <sup>Ac</sup>	4,28 ± 0,02 <sup>Ad</sup>	4,36 ± 0,01 <sup>Ab</sup>	4,27 ± 0,01 <sup>Ad</sup>
		4,44 ± 0,02 <sup>Aa</sup>	4,27 ± 0,01 <sup>Ad</sup>	4,22 ± 0,02 <sup>Ae</sup>	4,34 ± 0,02 <sup>Ac</sup>	4,28 ± 0,02 <sup>Ad</sup>	4,36 ± 0,01 <sup>Ab</sup>	4,27 ± 0,01 <sup>Ad</sup>
Acidity (%)	PP	0,48 ± 0,01 <sup>Bd</sup>	0,57 ± 0,06 <sup>Abc</sup>	0,58 ± 0,05 <sup>Aab</sup>	0,50 ± 0,07 <sup>Ac</sup>	0,6 ± 0,04 <sup>Aab</sup>	0,61 ± 0,04 <sup>Aab</sup>	0,66 ± 0,02 <sup>Aa</sup>
		0,48 ± 0,01 <sup>Bd</sup>	0,57 ± 0,06 <sup>Abc</sup>	0,58 ± 0,05 <sup>Aab</sup>	0,50 ± 0,07 <sup>Ac</sup>	0,6 ± 0,04 <sup>Aab</sup>	0,61 ± 0,04 <sup>Aab</sup>	0,66 ± 0,02 <sup>Aa</sup>
	Glass	0,56 ± 0,06 <sup>Ab</sup>	0,57 ± 0,03 <sup>Ab</sup>	0,59 ± 0,01 <sup>Aab</sup>	0,47 ± 0,09 <sup>Ac</sup>	0,54 ± 0,06 <sup>Bbc</sup>	0,58 ± 0,07 <sup>Ab</sup>	0,66 ± 0,01 <sup>Aa</sup>
		0,56 ± 0,06 <sup>Ab</sup>	0,57 ± 0,03 <sup>Ab</sup>	0,59 ± 0,01 <sup>Aab</sup>	0,47 ± 0,09 <sup>Ac</sup>	0,54 ± 0,06 <sup>Bbc</sup>	0,58 ± 0,07 <sup>Ab</sup>	0,66 ± 0,01 <sup>Aa</sup>
Moisture (%)	PP	25,36 ± 2,09 <sup>Ac</sup>	28,13 ± 0,77 <sup>Aa</sup>	27,60 ± 0,67 <sup>Aab</sup>	27,42 ± 0,71 <sup>Aa</sup>	28,03 ± 0,44 <sup>Aab</sup>	27,01 ± 1,19 <sup>Aab</sup>	26,34 ± 0,7 <sup>Bbc</sup>
		25,36 ± 2,09 <sup>Ac</sup>	28,13 ± 0,77 <sup>Aa</sup>	27,60 ± 0,67 <sup>Aab</sup>	27,42 ± 0,71 <sup>Aa</sup>	28,03 ± 0,44 <sup>Aab</sup>	27,01 ± 1,19 <sup>Aab</sup>	26,34 ± 0,7 <sup>Bbc</sup>
	Glass	26,04 ± 0,56 <sup>Ac</sup>	28,92 ± 1,08 <sup>Aa</sup>	27,95 ± 1,2 <sup>Aab</sup>	27,42 ± 1,04 <sup>Abc</sup>	28,03 ± 0,8 <sup>Aab</sup>	27,62 ± 1,07 <sup>Aab</sup>	27,45 ± 0,16 <sup>Abc</sup>
		26,04 ± 0,56 <sup>Ac</sup>	28,92 ± 1,08 <sup>Aa</sup>	27,95 ± 1,2 <sup>Aab</sup>	27,42 ± 1,04 <sup>Abc</sup>	28,03 ± 0,8 <sup>Aab</sup>	27,62 ± 1,07 <sup>Aab</sup>	27,45 ± 0,16 <sup>Abc</sup>
Total Sugars (%)	PP	22,08 ± 4,42 <sup>Aab</sup>	33,48 ± 9,8 <sup>Aa</sup>	37,77 ± 25,8 <sup>Aa</sup>	20,58 ± 9,92 <sup>Bab</sup>	6,75 ± 1,82 <sup>Ab</sup>	36,27 ± 8,09 <sup>Aa</sup>	32,39 ± 10,06 <sup>Aa</sup>
		22,08 ± 4,42 <sup>Aab</sup>	33,48 ± 9,8 <sup>Aa</sup>	37,77 ± 25,8 <sup>Aa</sup>	20,58 ± 9,92 <sup>Bab</sup>	6,75 ± 1,82 <sup>Ab</sup>	36,27 ± 8,09 <sup>Aa</sup>	32,39 ± 10,06 <sup>Aa</sup>
	Glass	20,67 ± 4,08 <sup>Ab</sup>	11,53 ± 5,45 <sup>Bb</sup>	40,78 ± 12,82 <sup>Aa</sup>	41,58 ± 23,63 <sup>Aa</sup>	16,8 ± 5,41 <sup>Ab</sup>	28,92 ± 13,18 <sup>Aab</sup>	28,92 ± 14,1 <sup>Aab</sup>
		20,67 ± 4,08 <sup>Ab</sup>	11,53 ± 5,45 <sup>Bb</sup>	40,78 ± 12,82 <sup>Aa</sup>	41,58 ± 23,63 <sup>Aa</sup>	16,8 ± 5,41 <sup>Ab</sup>	28,92 ± 13,18 <sup>Aab</sup>	28,92 ± 14,1 <sup>Aab</sup>

Means ± standard deviation followed by the same capital letter in the column and lowercase in the row do not differ by Tukey test at 5% probability. PP: Polypropylene. Source: Research data.

According to the data shown in Table 1, the pH tended to decrease during storage. Regarding packaging, there was a significant difference ( $p \leq 0.05$ ) in pH at 60 and 150 days, and the pH of the jelly in the PP packaging was lower than that in the glass packaging. Several studies that analyzed the stability of jellies during storage also observed a reduction in pH over time (Hossen et al., 2009; Safdar et al., 2012; Arévalo- Pinedo et al., 2013). In this study, the acidity of the jellies tended to increase throughout the storage period ( $p \leq 0.05$ ), and the packaging significantly affected acidity ( $p \leq 0.05$ ) at 0 and 120 days. The increase in acidity is related to the reduction of pH, as discussed above. Acidity is an important parameter for the formation and stability of the gel, and it must be 0.3–0.8%, because above this value, syneresis may occur (Assis et al., 2007; Dias et al., 2011). Despite the increased acidity, the values remained within the recommended range for avoiding syneresis. It was observed by Haq & Darakshan (2014) an increase in acidity in apricot jellies over 60 days of storage,

resulting from the formation of acidic compounds due to the degradation of organic compounds.

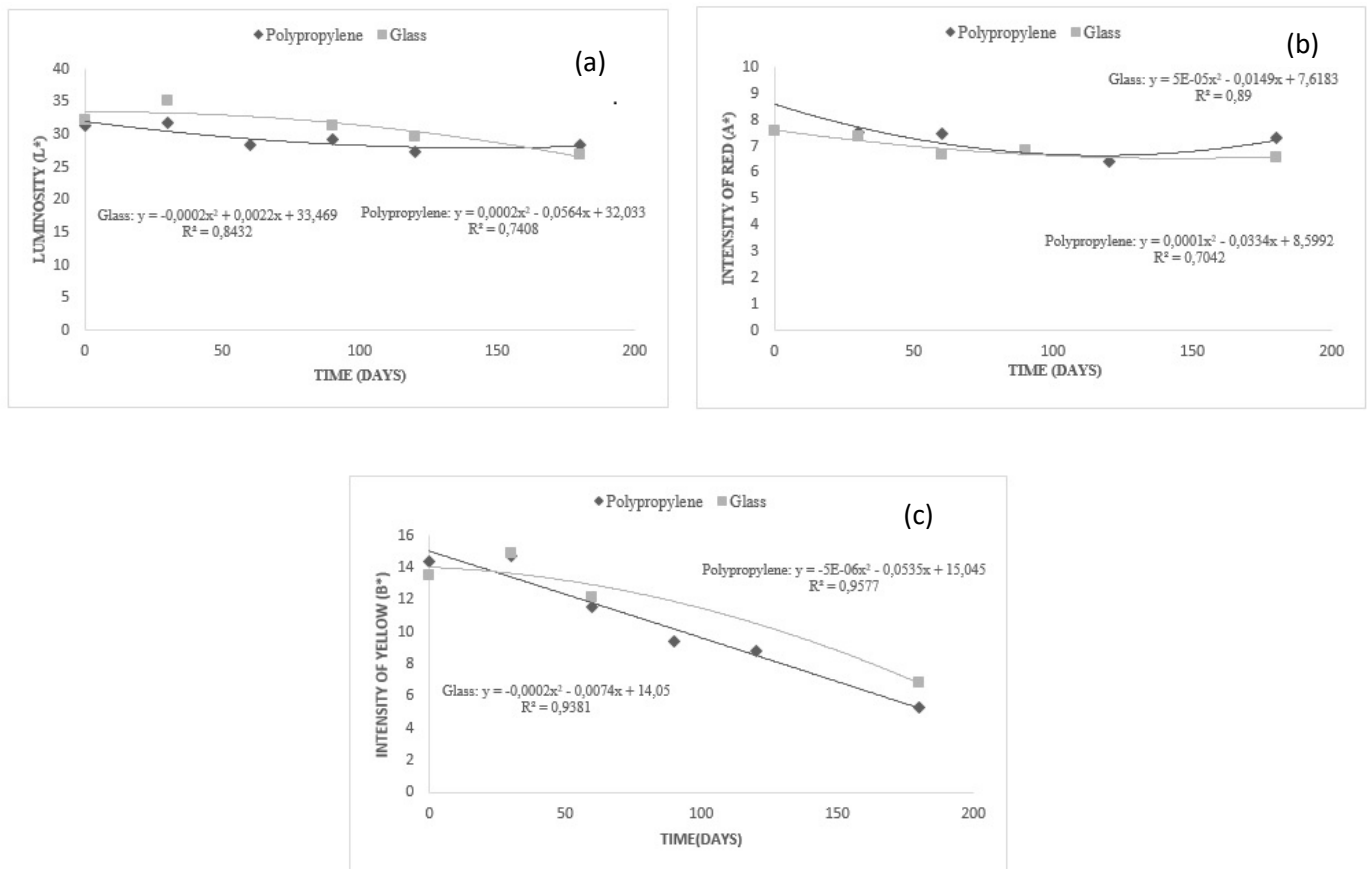
The moisture of the jellies was significantly increased ( $p \leq 0.05$ ) at 30 days of storage, but then stabilized and remained at this level until the end of the storage period (Table 1). Regarding packaging, a significant difference ( $p \leq 0.05$ ) was only observed at the end of storage period, and the highest average moisture content was observed in the glass packaging. As glass packaging is chemically inert, and its closure does not compromise this feature, the higher moisture content at the end of the storage period cannot be attributed to packaging failures. Therefore, the increase in moisture observed at the end of storage period may have been caused by sugar hydrolysis, which releases water molecules (Cunha, 2019). These data corroborate the results of Martins et al. (2015) in a study on the stability of cajá jellies during storage. In their study, jellies stored in glass containers showed increased moisture with storage (150 days). The authors stated that was due to interruption of the gel structure formed by pectin, sugar, and water, favoring the release of water and a consequent increase in moisture.

The total sugar content of the jellies tended to remain stable during storage; however, there was a significant reduction at 120 days and a subsequent increase at 150 and 180 days in both packaging ( $p \leq 0.05$ ) (Table 1). These data are similar to those collected in studies by Arévalo-Pinedo et al. (2013) in which the stability of araticum jellies. In these studies, there was no significant change in the total sugar content during storage.

### **3.2 Physical evaluation of low-calorie orange jellies**

Figure 1 show the effects of storage on the luminosity ( $L^*$ ) (a), red intensity ( $a^*$ ) (b) and yellow intensity ( $b^*$ ) (c) of low-calorie orange jellies in different packaging.

**Figure 1.** Effect of storage on the luminosity ( $L^*$ ) (a), intensity of red ( $a^*$ ) (b) and intensity of yellow ( $b^*$ ) (c) of low calorie orange jellies packed in different packaging.



Source: Research data.

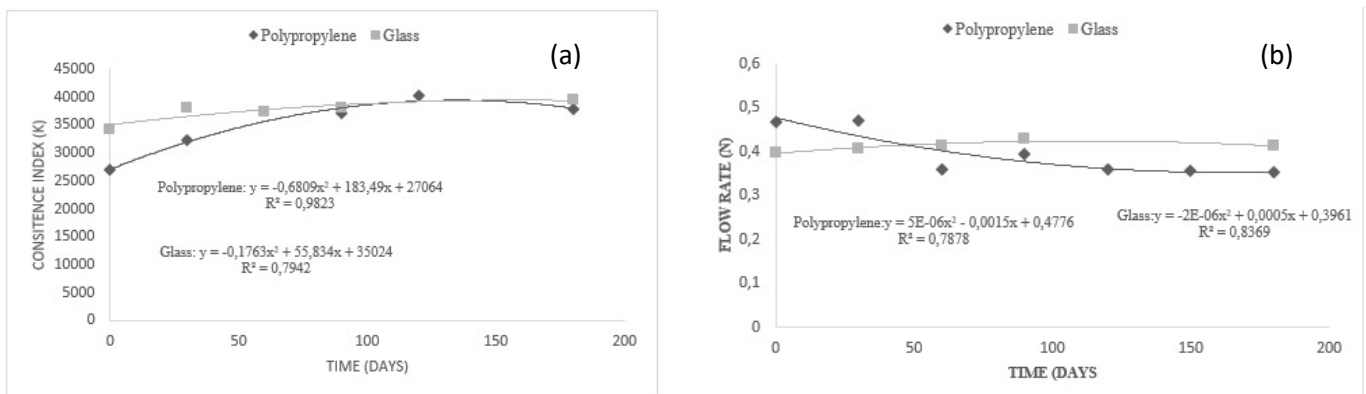
The luminosity ( $L^*$ ) (Figure 1a) of the jellies was stable, but tended to decrease in both packaging. Jellies stored in PP packaging had lower  $L^*$  values than those stored in glass packaging, indicating that the jelly stored in PP was darker. The PP packaging has a lower oxygen barrier, which favors the oxidation of vitamin C, producing hydroxymethylfurfural and furfural, which generate melanoidins, compounds that confer a dark coloration (Gava et al., 2008; Gliemmo et al., 2009). Was reported by Damiani et al. (2012a) a reduction in  $L^*$  values during the storage of araçá jam packed in glass containers, which the authors attributed to the Maillard reaction and the formation of hydroxymethylfurfural due to the oxidation of vitamin C.

Red intensity ( $a^*$ ) was generally stable during storage, but showed a tendency to increase ( $p \leq 0.05$ ) at the end of the storage period (Figure 1b). Red intensity values tended to increase during storage in both packaging, probably due to non-enzymatic darkening reactions that can generate red pigments (Gliemmo et al., 2009).

The yellow intensity ( $b^*$ ) values were close to +b (Figure 1c), which is characteristic of the fruit pulp from which the jelly was formulated (Ramalhosa et al., 2017). During storage, the  $b^*$  of the jellies in both packaging tended to decrease. This reduction may be due to degradation of the compounds in the fruit base from which the jelly was formulated. Damiani et al. (2012b) and Oliveira et al. (2014) also observed a reduction in  $b^*$  values in araçá and umbu-cajá jellies, respectively, during storage.

Figure 2 show the effects of the glass and PP packaging on the consistency (k) and flow rate (n) of the low-calorie orange jellies. The consistency index was not affected by the packaging (Figure 2a), as the consistency index of the jellies in both packaging was stable, but showed a tendency to increase over time. An increase in consistency during storage was also reported by Vahedi et al. (2008), who studied the quality of a yogurt formulation during storage, and Garrido et al. (2015), who studied the rheological parameters, color, and acceptance of apple jelly.

**Figure 2.** Effect of storage on the consistency index (k) (a) and flow rate (n) (b) of low-calorie orange jellies packed in different packaging.



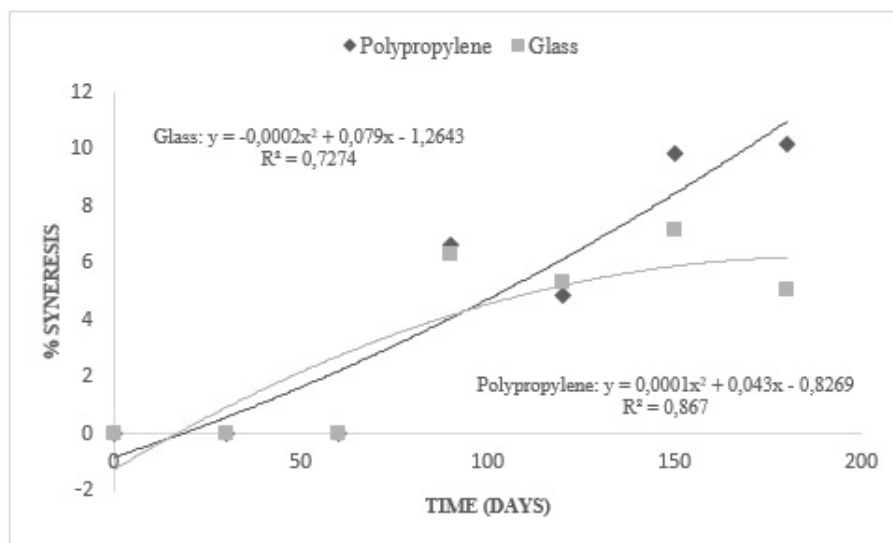
Source: Research data.

The flow rate (Figure 2b) is a measure of the rheological behavior of a product. The results showed that the flow rate values of the jellies in both packaging remained  $<1$  during the entire storage period, indicating that the non-Newtonian behavior and pseudoplastic characteristic of the jellies did not change during 180 days of storage. In addition, the packaging did not significantly influence ( $p > 0.05$ ) the rheological behavior; however, there was difference in storage time in the PP packaging ( $p \leq 0.05$ ). At 60 days of storage, there was a tendency towards reduced flow rates in the jellies stored in PP packaging. This reduction in the flow rate may be due to a reduction in pH, because reductions in pH favor

reduced electrostatic repulsion between pectin molecules due to a decrease in the dissociation of carboxylic groups, favoring an increase in the possibility of contact between these molecules and consequent increase of gel stiffness (Gava et al., 2008).

Figure 3 show the occurrence of syneresis in low-calorie orange jellies in relation to the packaging used (PP and glass) over the storage period (180 days).

**Figure 3.** Evaluation of syneresis (%) of low-calorie orange jellies packed in different packaging.



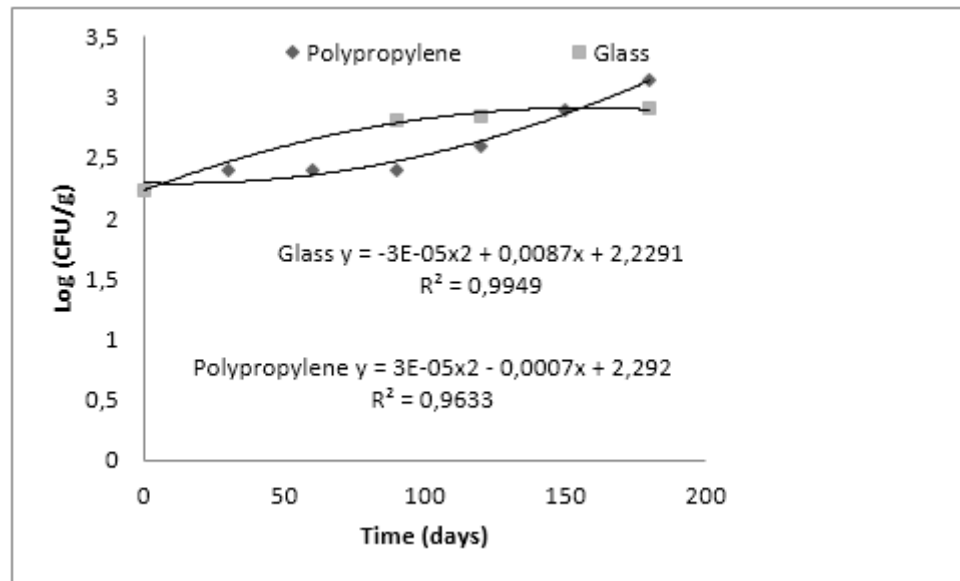
Source: Research data.

Syneresis occurred after 90 days of storage (Figure 3). Several factors may be involved in the occurrence of syneresis, including the low pH, high acidity, and excess invert sugar (Teles et al., 2017). Despite the occurrence of syneresis after 90 days, the values were not significant ( $p > 0.05$ ). This limited syneresis may be due to the use of hydrocolloids, which favor the formation of a more rigid and more stable gel. Khouryieh et al. (2005) evaluated the influence of different hydrocolloids, alone and in combination, on gel stability in low-sugar jellies and observed that a combination of hydrocolloids favored a reduction in syneresis during storage.

### 3.3 Microbiological evaluation

The Figure 4 show the molds and yeasts counts in the low-calorie orange jellies as a function of storage time and packaging.

**Figure 4.** Mold and yeast counts (log CFU / g) of low-calorie orange jellies packed in different packaging.



Source: Research data.

There was molds and yeasts growth in the jellies in both packaging during storage; however, there was significantly ( $p \leq 0.05$ ) lower growth in the glass packaging over time. Despite the molds and yeasts growth, the values were within the acceptable range under Brazilian law (maximum,  $10^4$  CFU/g) (Brazil, 2001). Several factors may favor the growth of molds and yeasts during storage, and the main ones are acidic pH, high moisture, high storage temperatures, the presence of oxygen, the chemical composition of the food, and the added sugars, which are used as energy source for microbial growth (Franco & Landgraf, 2001; Azeredo, 2012). The PP packaging has a lower oxygen barrier (Jorge, 2013) compared to the glass packaging, which favors the growth of molds and yeasts, since most of these microorganisms are aerobic (Franco & Landgraf, 2001). Effect of oxygen-barrier and barrier-free packaging on the characteristics of orange juice that was unpasteurized, pasteurized at 66 °C for 10 s, and pasteurized at 90 °C for 60 s was evaluated by Sadler et al. (1992). The authors observed that, for pasteurized juices, the oxygen-barrier packaging improved the microbiological stability of orange juice.

### 3.4 Bioactive compounds and antioxidant activity

Table 2 presents the average results for the bioactive compounds and the antioxidant activity of low-calorie orange jellies stored in different packaging throughout the storage.

**Table 2.** Evaluation of bioactive compounds and the antioxidant activity of low-calorie orange jellies in relation to the packaging (polypropylene and glass) and storage time (180 days).

Variables	Packaging	Time (days)							SEM <sup>†</sup>
		0	30	60	90	120	150	180	
<b>Antioxidant activity - DPPH (EC<sub>50</sub> g jelly/g DPPH)</b>	<b>PP</b>	39085.8 <sup>Ab</sup>	30369.9 <sup>Abc</sup>	35977.8 <sup>Abc</sup>	60106.7 <sup>Aa</sup>	18924.1 <sup>Ac</sup>	3739.4 <sup>Ad</sup>	11500.8 <sup>Ad</sup>	2879.24
	<b>Glass</b>	43094.8 <sup>Aa</sup>	30922.8 <sup>Aabc</sup>	34508.7 <sup>Aab</sup>	45355.5 <sup>Ba</sup>	21814.7 <sup>Abcd</sup>	4413.0 <sup>Ad</sup>	13163.4 <sup>Ac</sup>	2141.65
<b>Antioxidant activity - ABTS (µMtrolox/g de jelly)</b>	<b>PP</b>	1.32 <sup>Aa</sup>	1.01 <sup>Aa</sup>	2.37 <sup>Aa</sup>	1.58 <sup>Aa</sup>	2.35 <sup>Aa</sup>	1.04 <sup>Aa</sup>	1.51 <sup>Aa</sup>	0.17
	<b>Glass</b>	1.98 <sup>Aa</sup>	0.94 <sup>Aa</sup>	1.34 <sup>Aa</sup>	1.45 <sup>Aa</sup>	2.01 <sup>Aa</sup>	1.42 <sup>Aa</sup>	1.41 <sup>Aa</sup>	0.11
<b>Antioxidant activity - β-carotene (% Protection)</b>	<b>PP</b>	11.28 <sup>Ac</sup>	51.96 <sup>Ba</sup>	61.38 <sup>Aa</sup>	28.13 <sup>Ab</sup>	16.27 <sup>Abc</sup>	20.93 <sup>Abc</sup>	11.01 <sup>Ac</sup>	2.93
	<b>Glass</b>	10.77 <sup>Abc</sup>	65.17 <sup>Aa</sup>	52.71 <sup>Aa</sup>	25.57 <sup>Ab</sup>	17.15 <sup>Abc</sup>	18.62 <sup>Abc</sup>	5.36 <sup>Ac</sup>	2.83
<b>Total Phenolics (mgAGE/g jelly)</b>	<b>PP</b>	0.33 <sup>Ab</sup>	0.26 <sup>Ac</sup>	0.25 <sup>Bc</sup>	0.72 <sup>Aa</sup>	0.33 <sup>Bb</sup>	0.26 <sup>Ac</sup>	0.35 <sup>Ab</sup>	0.02
	<b>Glass</b>	0.34 <sup>Ab</sup>	0.25 <sup>Ac</sup>	0.28 <sup>Ac</sup>	0.62 <sup>Ba</sup>	0.37 <sup>Ab</sup>	0.23 <sup>Ad</sup>	0.35 <sup>Ab</sup>	0.02
<b>Vitamin C (mg/100g)</b>	<b>PP</b>	147.4 <sup>Aa</sup>	107.1 <sup>Babc</sup>	76.64 <sup>Abcd</sup>	50.92 <sup>Ad</sup>	62.86 <sup>Ac</sup>	119.4 <sup>Aab</sup>	80.39 <sup>Bbcd</sup>	5.29
	<b>Glass</b>	130.6 <sup>Aab</sup>	147.2 <sup>Aa</sup>	80.62 <sup>Abcd</sup>	50.67 <sup>Ad</sup>	67.20 <sup>Ac</sup>	103.6 <sup>Aabc</sup>	115.3 <sup>Aabc</sup>	6.59

Means followed by the same capital letter in the column and lowercase in the row do not differ from each other by the Tukey test at 5% probability. <sup>†</sup>Standard Error Mean (SEM). PP: polypropylene. Source: Research data.

The antioxidant activity of low-calorie orange jellies by DPPH displayed a tendency to increase during storage since lower values of EC<sub>50</sub> are equivalent to higher antioxidant activity (Table 2). There was a significant difference (p≤0.05) in relation to the packaging only at 90 days of storage, and the highest averages were found in the glass packaging. The



factor that may have assisted the increased antioxidant activity in jellies was the degradation of fruit compounds, and the consequent formation of degradation products that may have antioxidant activity (Shinwari & Rao, 2018). The study by Damiani et al. (2012b), which evaluated the antioxidant potential of araça jelly during storage, also showed an increase in antioxidant activity up to 8 months of storage, followed by a decrease up to 12 months of storage.

Regarding the other methodologies used to evaluate the antioxidant activity, it can be observed that the methodology proposed by the reduction of the ABTS•+ radical did not present a significant difference in relation to storage time and packaging used ( $p > 0.05$ ). Moreover, the methodology proposed by the inhibition of lipid peroxidation in the  $\beta$ -carotene/linoleic acid system demonstrated stability at the beginning and end of storage, with small variations over time. These methodologies are limiting due to the selectivity to certain compounds (Hassimoto et al., 2005). The ABTS•+ methodology presents differences in the incubation time and low selectivity in the reaction with hydrogen donor atoms, while the methodology proposed by the percentage of lipid peroxidation is more sensitive to lipophilic antioxidant compounds. Thus, this may be the cause of the difference in the results when compared to the methodology of DPPH (Hassimoto et al., 2005).

The phenolic content present in the jellies were observed to be stable with a tendency to decrease during storage. The packaging influenced changes significantly ( $p \leq 0.05$ ) in the phenolic content at 60, 90, and 120 days of storage, with the lowest averages observed in jellies stored in PP packaging. The use of low methoxylated pectin (LMP) in jellies may be related to the retention of phenolic content, as reported in studies with low-calorie and conventional jellies, the mechanism for which remains to be elucidated (Shinwari & Rao, 2018). The studies of Damiani et al. (2012a), Abolila et al. (2015), and Kamiloglu et al. (2015) presented a reduction in the amount of total phenolic content during storage. This may have happened due of the high instability of these content at temperatures between 20 to 40 °C. These studies used ambient temperature (25 °C), corroborating with the ambient conditions of this study. Non-enzymatic reactions, which occur when cell structures are interrupted, favor the degradation of phenolic content (Patras et al., 2011).

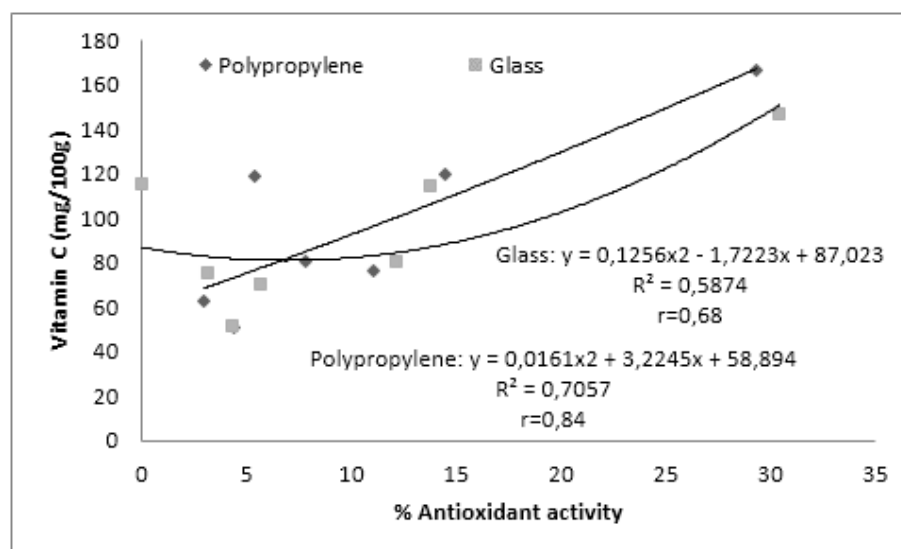
The results for vitamin C demonstrated reduced values during storage. The packaging used was observed to significantly influence ( $p \leq 0.05$ ) the vitamin C values from the 30<sup>th</sup> day of storage, with a loss of 45.5% noted in the PP packaging, whereas in the glass packaging, the loss was noted to be 11.72%. Several factors can trigger vitamin C degradation, such as the presence of oxygen, light, high processing, pH, acidity, enzymes, and/or storage

temperatures among others. The presence of light and the storage temperature (25 °C) may have favored the reduction of vitamin C during storage in both packaging. However, the lower oxygen barrier of the PP packaging may have been the factor that triggered the most significant loss of vitamin C. The study by Carneiro et al. (2016) demonstrated a reduction of vitamin C in blackberry jellies after 90 days of storage, as being higher in PP packaging than in glass packaging. The authors related this finding with the greater permeability of PP packaging to oxygen, favoring lower retention of vitamin C.

The classification of fruits and fruit products based on vitamin C content is as follows: low (<30 mg/100 g), medium (30-50 mg/100 g) and high (> 50 mg/100 g) (Souza et al., 2012). Thus, despite the losses of vitamin C during storage, low-calorie orange jellies can be classified as high in vitamin C, as well as favoring adequate intake of vitamin C with the consumption of 100 g of jelly, according to vitamin C recommendation for adults (90 mg/day for men and 75 mg/day for women) (Cuppari, 2005).

There was a positive correlation ( $r = 0.84$ ) observed between the antioxidant activity and amount of vitamin C, with regard to PP packaging and poor correlation was observed for glass packaging ( $r = 0.68$ ), as shown in Figure 5.

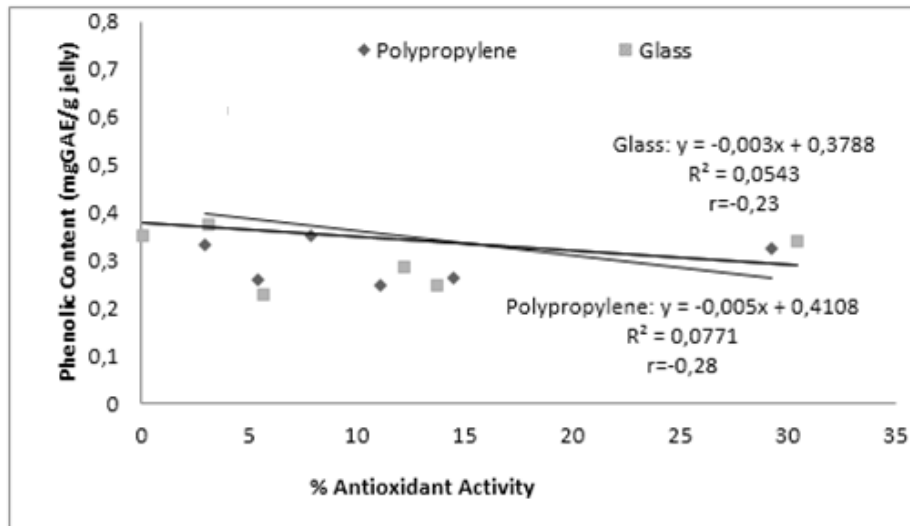
**Figure 5.** Regression analysis from the correlation calculations between antioxidant activity and vitamin C content in low-calorie orange jellies in relation to storage time and packaging used.



Source: Research data.

In relation to the antioxidant activity and phenolic content, there was no correlation, since the results were negative ( $r = -0.28$  for PP packaging and  $r = -0.23$  for glass packaging), as shown in Figure 6.

**Figure 6.** Regression analysis from the correlation calculations between antioxidant activity and phenolic content in low-calorie orange jellies in relation to storage time and packaging used.



Source: Research data.

Therefore, vitamin C was determined to be the compound that most contributed to the antioxidant activity of low-calorie orange jellies stored in PP packaging, corroborating the study by Souza et al. (2012) that, when determining the antioxidant activity, chemical composition and bioactive compounds of fruits of the cerrado, verified a positive correlation between vitamin C and the antioxidant activity of fruits.

#### 4. Final Considerations

The pH, acidity, and mold and yeast growth in fruit jellies were influenced by storage time. Although mold and yeasts were observed, the counts were in compliance with Brazilian law. Syneresis occurred after 90 days of storage; however, it was not significantly related to storage time or packaging.

The packaging and time of storage influenced the content of bioactive compounds at the end of storage. There was a tendency to increase the antioxidant activity, determined by

the DPPH method, which could not be verified by the ABTS•+ and  $\beta$ -carotene/linoleic acid methodologies.

There was a reduction of vitamin C observed throughout the storage, accompanied with exhibition of a positive correlation with the antioxidant activity in the polypropylene packaging, indicating that this compound influenced the antioxidant activity in this packaging.

It was demonstrated that the stability of total phenolic content reduced substantially in PP packaging. There was no correlation of these compounds with the antioxidant activity.

It can be concluded, therefore, that glass packaging was more favorable to maintain the physicochemical, physical, and microbiological character and bioactive compounds stability at the end of the storage since the most significant changes in these compounds occurred in the PP packaging. Thus, campaigns should be carried out with the fruit jelly processing industries to highlight the importance of storing these products in glass packaging, in order to promote a product with greater nutritional value, safe and with greater stability.

In the future, it is suggested that sensory tests be carried out over the storage period in order to verify product acceptability by consumers.

## Acknowledgments

The authors gratefully acknowledge the UFOP and FAPEMIG (APQ-02047-14) for their financial assistance in the study. The authors also acknowledge Nutramax® for supporting the study by donating sweeteners.

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