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EVALUATION OF ANTIOXIDANT POTENTIAL OF ORGANIC VEGETABLES DURING COLD STORAGE

AVALIAÇÃO DO POTÊNCIAL ANTIOXIDANTE DE HORTALIÇAS ORGÂNICAS DURANTE O ARMAZENAMENTO A FRIO

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ABSTRACT

The relation between an antioxidant-rich diet with protection against some diseases such as Alzheimer's and cardiovascular diseases has increased research on the evaluation of these components in food. Another factor related to some diseases is the consumption of food produced from conventional agriculture with the excessive use of agrochemicals and the search for food free of pesticides increases the demand for products grown in organic systems. Aiming to relate these two themes, the present study aimed to produce a kit of organic vegetables and evaluate antioxidant activity during storage at reduced temperatures. The results indicate a sudden drop in antioxidant activity and phenolic compound concentration in the first month of storage.

Keywords: organic vegetables, antioxidant activity.

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RESUMO

A relação da alimentação rica em antioxidantes com a proteção contra algumas doenças como Alzheimer e doenças cardiovasculares, faz com que a pesquisa relacionada à avaliação desses componentes em alimentos aumente. Outro fator também já relacionado com algumas doenças é o consumo de alimentos produzidos a partir da agricultura convencional com o uso excessivo de agrotóxicos, a busca por alimentos isentos de agrotóxicos faz crescer a demanda dos produtos cultivados em sistema orgânico. Buscando relacionar esses dois temas, o estudo desenvolvido teve como objetivo produzir um kit de hortaliças orgânicas e avaliar a atividade antioxidante durante o armazenamento em temperaturas reduzidas. Os resultados obtidos indicam uma queda brusca na atividade antioxidante e na concentração dos compostos fenólicos já no primeiro mês de armazenamento.

Palavras-chave: *hortaliças orgânicas, atividade antioxidante.*

INTRODUCTION

The search for better quality foods has constantly grown, as has the concern for the risks they may pose. It is from these principles that organic food production and composition evaluation have gained more prominence.

For Sediyama, Santos, and Lima (2014), organic agriculture appears not only as an alternative form for the current conventional system, but also as a strong basis for a paradigm shift in the relationship between society and agriculture. The application of social, ecological, and environmental issues in dealing with agriculture is the main differential of this system, since it balances relations and their sustainability in time and space.

According to Darolt (2003), the quality of food products depends on many factors, in addition to the form of production (organic or conventional), which may affect the quality of a food, including genetic factors (varieties), climate, soil conditions, postharvest- storage, among others. The evaluation of a food from the centesimal composition allows us to know the majority of the components present through classification in homogeneous groups. However, evaluation of phytochemical compounds is increasingly being developed, since phytochemical-rich diets have been highlighted, according to Hyson (2002), as a protective factor for diseases.

Podsedek (2007) reported that phytochemical compounds include antioxidant substances, which reduce the risk of a variety of chronic diseases, including atherosclerosis and cancer, due to the absorption of free radicals, inhibiting the initiation chain or interrupting the propagation chain of the oxidative reactions promoted by the radicals formed in the human organism.

Therefore, the overall objective of this study was to evaluate the quality of a kit with five varieties of organic vegetables (zucchini, green corn, broccoli, carrots, and kale) during a given period of storage at low temperatures. For the evaluation, the kits were produced from blanched and unblanched vegetables in order to compare the data obtained.

1. LITERATURE REVIEW

1.1 ANTIOXIDANT ACTIVITY

The formation of free radicals in the human body is responsible for causing damage and leading to an onset of diseases and occurs in aerobic cells through respiration and oxidative reactions (SIKORA et al., 2008), or by being absorbed through exogenous factors such as diet, medication, smoking, car exhaust fumes, strenuous physical activity, among others (GIADA;

MANCINI, 2006). Protection against the effects of free radicals is provided by an antioxidant system consisting of an array of various fat-soluble (vitamin E; carotenoid), water-soluble (ascorbic acid; glutatinone), and enzymatic (glutatinone peroxidase; superoxide dismutase; catalase) components (McLEAN et al., 2005).

Fat-soluble and water-soluble components are present in vegetables commonly consumed daily (GIADA; MANCINI, 2006). Among the compounds that develop antioxidant activity, phenolic compounds, carotenes, and ascorbic acid (vitamin C) are present in large quantities in many vegetables and fruit part of daily diets.

1.2 PHENOLIC COMPOUNDS

These compounds have hydroxyl groups attached in the aromatic rings of their chemical structures. According to Baena (2015), their presence in vegetables is a result of their use in growth, production, and defense against parasites and predators, in addition to being responsible for the coloring of some vegetables.

The antioxidant action of these compounds is due to their ability to donate hydrogen or electrons and also produce stable intermediate radicals, which prevents the oxidation of various food ingredients (BRAND-WILLIAMS; CUVELIER; BERSET, 1995). For Silva et. al. (2010), the beneficial action of phenolic compounds in human health has been related to their anti-inflammatory activity and the activity that prevents not only the agglomeration of blood platelets, but also the action of free radicals in the body. Since they protect molecules such as DNA, they may abort some carcinogenic processes.

1.3 CAROTENOID COMPOUNDS

Baena (2015) defines carotenoid compounds as substances that present long acyclic chains with a variable series of double bonds in the central region of the molecule, presenting cyclic structures at one or both ends, which may present various arrangements with oxygenated groups. Moreover, in plant physiology, carotenoids are related to photosynthesis and photoprotection of plants due to their ability to sequester reactive oxygen species formed from exposure to sunlight and their role in the coloration of some vegetables (BAENA, 2015).

As indicated by Sousa et. al (2007), carotenoid antioxidant activity is caused by the ability to displace unpaired electrons through the conjugate double bond structure, and three proposals for mechanisms for the reaction of free radicals with carotenoids are reported in the literature.

1.4 ASCORBIC ACID

Ascorbic acid is usually called vitamin C and, according to Pereira (2008), has a chemical formula $C_6H_8O_6$ and a polar molecule with four hydroxyl groups (OH) in its chemical structure. This substance belongs to the organic group called lactones, which is composed of carboxylic acids that turn into cyclic esters, i.e. closed-chain esters that lost water spontaneously.

Vitamin C can be found in plant tissues and is synthesized by most mammals, except primates, guinea pigs, and some bat species (COULTATE, 2004). The antioxidant effect of this substance works in many ways, including the removal of oxygen, which prevents the oxidation of sensitive constituents and the regeneration of antioxidants, in addition to acting together with complexing agents and or in the reduction of undesirable oxidation products (RAMALHO, 2005).

2. METHODOLOGY

To develop the frozen vegetables kit, we used organic vegetables provided by the Central Cooperative of Family Agriculture Ltda (UNICOOPER), Santa Rosa - RS. The study was carried out at the Nutrition Laboratories of the Universidade Regional do Noroeste do Estado do Rio Grande do Sul (UNIJUI), Ijuí-RS .

Each vegetable was properly washed and sanitized in chlorinated water. After selection, peeling, and cutting, blanching was performed at 80 ºC with half of each variety of vegetables.

Blanching is a heat treatment that can be applied to fresh fruit and vegetables prior to freezing, drying or canning. Its main purpose is to inactivate enzymes that normally cause browning, nutrient degradation and/or food spoilage during preparation. Blanching is not considered a preservation process if used alone, but rather a treatment that precedes other methods providing the food with more durability and quality of its sensory characteristics (FELLOWS, 2006).

The percentages used were chayote (30%), carrot (20%), green corn (25%), zucchini (10%), broccoli (10%), and collard greens (5%). For the monthly evaluation, 5 kits of 200 grams each (Figure 1) with blanched vegetables and 5 with unblanched vegetables were produced, all vacuum packed, frozen, and stored at -18 ºC for later physicochemical evaluation in the following 5 months.

For analyses, a blanched and unblanched vegetable kit were removed from the freezer each month, unpacked, shredded with the aid of a mixer, homogenized, and placed in plastic containers.

Figure 1: Organic vegetable kit provided by the Cooperativa Central da Agricultura Familiar Ltda (UNICOOPER), Santa Rosa - RS, produced in 2018.

Source: Authors

2.1 METHODOLOGY TO DETERMINE PHENOLIC COMPOUNDS

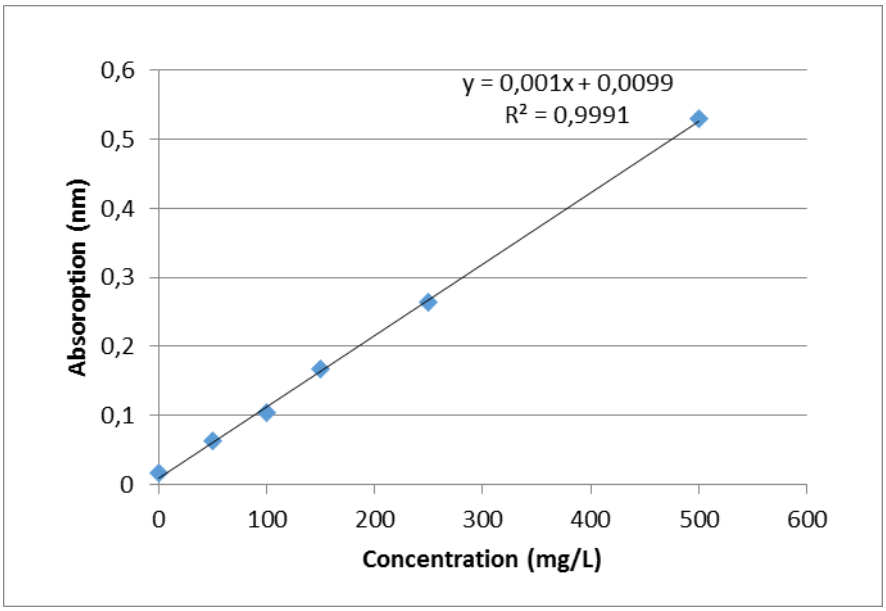
The content of total phenolic compounds was determined according to the method adapted by Rufino *et al*. (2007). For development of the methodology, the extracts were produced with a 3-gram sample from each kit was used, transferred to 50-ml Falcon tubes with 10 mL of 70% acetone and 10 mL of 50% methanol. After homogenization, the mixture was kept at rest for 1 h for the phenolic compounds to migrate from the solid to the liquid phase.

After resting, the mixture was centrifuged for 20 min at 10.000 rpm and temperature of 5 °C. The obtained supernatant was filtered off and transferred to a 25-mL volumetric flask that was filled with distilled water. A 2.5 g/L gallic acid solution was used to build the standard

curve. For the curve points, other solutions at concentrations of 0, 50, 100, 150, 250, and 500 mg/L were produced.

With the sample extract and the curve points obtained, 0.1 mL of each concentration, 2 mL of distilled water, and 1 mL of Folin-Ciocalteu reagent were placed in different test tubes. After 3 min, 5 mL of 20% sodium carbonate and 5.4 mL of distilled water was added to obtain a final volume of 10 mL. After 30 minutes of resting in the dark, spectrophotometer readings were performed at 765 nanometers. The standard curve was made every month and according to the equation obtained from the curve, by considering the dilutions made, the concentration of phenolic compounds can be obtained. For visualization, the curve of the first month of freezing is represented in the figure below.

Figure 2: Standard curve of gallic acid from the first month of freezing for polyphenol analysis in the precooked organic vegetables kit (blanched) and the in natura organic vegetable kit, UNIJUI, Ijuí/RS, 2018.



Source: Authors

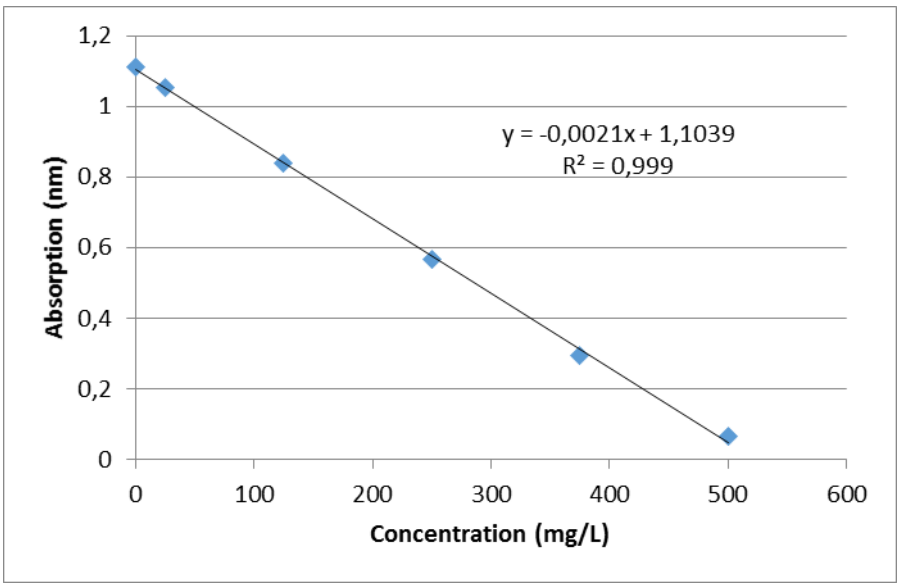
2.2 METHODOLOGY TO DETERMINE PHENOLIC COMPOUNDS

Antioxidant activity was analyzed by the ability to sequester DPPH radicals according to Brand-Williams, Cuvelier, and Berset (1995), hence, TROLOX (*6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid*) was used as standard antioxidant. In the development of this methodology, extracts from acetone 70% and methanol 50% were used to evaluate phenolic compounds, as these solvents have the ability to extract antioxidant substances from solid samples.

The 0.5 g/L solution of TROLOX in ethyl alcohol was used as standard to produce solutions with concentrations of 0, 25, 125, 250, 375, and 500 mg/L, which were used to construct the points of the standard curve. For the DPPH reagent (*2,2-difenil-1-picrilhidrazil*), the 0.24 g/L stock solution in methanol was initially prepared and from it another solution was prepared at the ratio of 1 ml stock solution for every 4.5 mL of methanol. According to the methodology, the DPPH solution used must present absorbance between 1.08 and 1.12 at the wavelength of 517 nanometers, and correction should be made by adding methanol or stock solution if necessary.

Evaluation of each curve a point of the samples was developed by adding 3.9 mL of the diluted DPPH solution by 0.1 mL of each point and each sample. Monthly curves were also developed for this analysis in order that, through the equation of the curve and with the dilutions made, it is possible to obtain the antioxidant concentrations in the samples. The curve made in the first month of freezing is shown below.

Figure 3: TROLOX standard curve of the first month of freezing for antioxidant analysis in the precooked organic vegetables kit (blanched) and the in natura organic vegetable kit, UNIJUI, Ijuí/RS, 2018.



Source: Authors

2.3 CAROTENOID DETERMINATION METHODOLOGY

Carotenoid content was analyzed following the method by Rodriguez-Amaya (1999). Extracts for analysis were obtained from a 5-gram sample of each mix and 20 mL of 70% acetone in Falcon tubes. After homogenization and 1-hour rest, the mixture was centrifuged at 10000 rpm for 10 minutes and the supernatant produced was filtered and reserved. This process of extraction with 70% acetone was repeated until the entire sample added to the tube did not show coloring.

The reading in the spectrophotometer should be performed with carotenoids dissolved in petroleum ether, therefore, the liquid-liquid extraction was carried out to trigger the migration of carotenoids from the acetone to the petroleum ether. This procedure was performed in an extraction funnel where 40 mL of petroleum ether was added in which the supernatant was gradually added, adding a little water and discarding the lower portion consisting of water and acetone.

After extraction, the carotenoid solution dissolved in ether was expanded in a 50-mL volumetric flask and read at a spectrophotometer wavelength of 453 nanometers. Carotenoid concentrations can be obtained from Equation 1 where: (V) is the volume of the extract obtained in mL, (A) is the absorbance measured at 453 nm, (ε) is the absorptivity of β-carotene at 453 nm (2592), and (m) the mass of the sample weighed in grams.

$$\text{Carotenoids } (\mu g/g \text{ sample}) = \frac{V(mL) \cdot A \cdot 10^4}{\varepsilon\ (mL/g.cm) \cdot m\ (g)} \quad (1)$$

2.4 METHODOLOGY FOR VITAMIN C DETERMINATION

Ascorbic acid levels were evaluated by the iodometric method indicated by the Adolfo Lutz Institute (2008) using potassium iodate in the presence of starch as an indicator.

For the evaluation, 10 grams of the sample with 20 mL of 20% sulfuric acid were used. The solution was filtered to facilitate viewing of the turning point and the obtained filtrate was expanded to 50 mL with water. Analysis is performed from the titration of the filtrate with potassium iodate and, in the presence of starch, the coloration darkens to the dark blue, which indicates the end of titration. The end result can be obtained from Equation 2 where: (V) is the volume of the potassium iodate solution used in mL and (m) the mass of the sample weighed in grams.

$$\text{Vitamina C } (\text{mg } 100g^{-1}) = \frac{V\ (mL).\ 100.\ 0{,}8806}{m\ (g)} \quad (2)$$

3. RESULTS AND DISCUSSION

The results of the analyses of vitamin C, antioxidants, polyphenols, and carotenoids carried out during the five-month research for the kits made from blanched vegetables is shown in Table 1.

Table 1: Vitamin C, Total Polyphenols, Total Carotenoids, and Antioxidants in a kit of pre-cooked (blanched) organic vegetables, values from the first freezing month to the fifth month. UNIJUI, Ijuí/RS, 2018.

Blanched	Vitamin C (mg $100g^{-1}$)	Antioxidants (µmol $100g^{-1}$)	Polyphenols (mg $100mg^{-1}$)	Carotenoids (µg g^{-1})
First month	8.78	881.08	506.72	36.1
Second month	8.53	171	38.9	32.67
Third month	8.41	109.76	27.37	30.21
Fourth month	7.59	77.72	41.16	21.48
Fifth month	6.12	72.95	18.1	14.8

Source: Authors

Table 2: Vitamin C, Total Polyphenols, Total Carotenoids, and Antioxidants in a kit of in natura organic vegetables, values from the first freezing month until the fifth month. UNIJUI, Ijuí/RS, 2018.

In natura	Vitamin C (mg $100g^{-1}$)	Antioxidants (µmol $100g^{-1}$)	Polyphenols (mg $100mg^{-1}$)	Carotenoids (µg g^{-1})
First month	8.17	886.6	530.26	25.2
Second month	7.25	146.03	46.56	19.55
Third month	6.19	113.08	31.26	18.18
Fourth month	4.82	83.69	35.78	18.57
Fifth month	4.53	77.06	17	17.66

Source: Authors

Vitamin C content varied during freezing, where the blanched sample starts with a content of 8.78mg/100g and varies to the lowest content of 6.12mg/100g, while the fresh sample has an initial content of 8.17mg/100g and decreases to 453mg/100g. During freezing, the fresh

sample showed higher losses of vitamin C compared to the blanched sample, which occurs since blanching causes the inactivation of enzymes (CORREIA et al., 2008).

Studies on the effects of different mango juice processing steps on vitamin C content have shown that cutting, defect removal, and pasteurization/blanching steps have more significant effects on the final vitamin C content than the freezing itself, because these steps occur in the presence of oxygen, which favors the oxidation of ascorbic acid (ALLAH and ZAKI, 1974).

The antioxidant activity of the samples begins in the first month of freezing with high values of 881.08 µmol/100g and 886.60 µmol/100g for blanched and fresh vegetables, respectively. These values decrease to 171.00 µmol/100g and 146.03 µmol/100g in the second month. For the following months, the decrease is less drastic, with the results in antioxidant activity being 72.95 µmol/100g in the blanched sample and 77.06 µmol/100g in the fresh sample.

In the polyphenol content, it is possible to observe the same drastic decrease in the second month. On the other hand, for the fresh sample, in the first month it was possible to observe a concentration of 530.26 mg/100g of polyphenols and 46.56mg/100g in the second month, revealing a 91% drop in polyphenol activity. As for the blanched sample, it varied from the first to the second month from 506.72mg/100g to 38.9mg/100g, reaching a value of 27.37mg/100g in the third month. The concentration at the end of the fifth month was 18.10 mg/100g in the blanched sample and 17.00 mg/100g in the fresh sample.

The carotenoid content in the samples ranged in the first month from 36.10 µg/g to 14.8 µg/g in the fifth month of the blanched sample. In the fresh sample, carotenoid content ranged from 25.20 µg/g to 17.66 µg/g, not presenting a sharp decrease in a given month, but a gradual decrease over the evaluation period.

In a study by Silva et al (2015) on the influence of blanching on total carotenoid content, the authors showed that heat utilization may reduce the amount of carotenoids. Here, we can see the initial content of blanched sample is greater than the fresh ones, which is not in agreement with the authors report. This is probably due to the greater decomposition of carotenoids in the in natura sample during the first month of storage in relation to the decomposition in the blanched sample.

CONCLUSION

The samples from the blanched and fresh kit showed no significant difference in nutritional quality throughout the five-month study. This suggests that both methods are good for freezing, although blanching is preferable, as it ensures better visual appearance to the product and maintains the color of the vegetables due to the denaturation of the enzymes that cause browning.

Regarding the concentration of phenolic compounds and antioxidant activity, it is plausible to conclude that consumption of the vegetable kit in the first month of storage ensures higher concentration of these components considering the fresh vegetable kit is rich in phenolic compounds and has higher antioxidant activity. For vitamin C and carotenoids, reduction is gradual and the blanched vegetable kit best preserves these properties.

Finally, all evaluated parameters show variations during freezing without major differences in the blanching process, since freezing already guarantees reduced speed of enzymatic reactions of the product.

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Contribution	[Author 1]	[Author 2]	[Author 3]
1. Definition of research problem	√		√
2. Development of hypotheses or research questions (empirical studies)	√		√
3. Development of theoretical propositions (theoretical work)	√	√	√
4. Theoretical foundation / Literature review	√	√	
5. Definition of methodological procedures			√
6. Data collection	√	√	
7. Statistical analysis	√	√	√
8. Analysis and interpretation of data	√	√	√
9. Critical revision of the manuscript	√	√	√
10. Manuscript writing	√	√	
11. Other (please specify)			