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EVALUATION OF THE ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC COMPOUNDS OF LYCHEE PULP BY DIFFERENT DEHYDRATION METHODS

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ABSTRACT

Fruits and vegetables are major sources of bioactive compounds included in human diet. The objective of the study was to evaluate the antioxidant activity and total phenolic compound content of lychee pulp. Hydroalcoholic extracts were obtained from the pulp (fresh, dried and freeze-dried), and analyzed by in vitro Folin-Ciocalteu, Fast Blue, ABTS$^+$, Beta Carotene and Phosphomolybdenum Complex assays. The color and water activity of the pulp submitted to each dehydration treatment were also evaluated. The results obtained in the determination of total phenolic content did not differ statistically ($p>0.05$) between dried and freeze-dried pulp. The values obtained for the antioxidant activity by the ABTS$^+$ method and by the phosphomolybdenum complex had a statistically significant difference. The data regarding the percentage of protection by the β-Carotene/Linoleic Acid method showed that the dried pulp did not differ statistically from the fresh and freeze-dried pulp. However, the freeze-dried pulp presented higher antioxidant activity by the ABTS$^+$ and Phosphomolybdenum Complex assays when compared to the other treatments (fresh and dried pulps).

Palavras-chave:
Compostos bioativos
Litchi chinensis
Liofilização
Secagem

AVALIAÇÃO DA ATIVIDADE ANTIOXIDANTE E COMPOSTOS FENÓLICOS TOTAIS DA POLPA DE LICHIA POR DIFERENTES MÉTODOS DE DESIDRATAÇÃO

RESUMO

As frutas e hortaliças são as principais fontes de compostos bioativos incluídos na dieta humana. O objetivo do estudo foi avaliar a atividade antioxidante e o teor de compostos fenólicos totais da polpa da lichia. Foram obtidos extratos hidroalcoólicos da polpa (fresca, seca e liofilizada), e analisados por meio de ensaios *in vitro Folin-Ciocalteu*, Fast Blue, ABTS$^+$, β-Caroteno e Complexo de Fosfomolibdênio. A cor e a atividade de água da polpa submetida a cada tratamento também foram avaliadas. Os resultados obtidos na determinação dos compostos fenólicos totais não diferiram estatisticamente ($p>0,05$) entre a polpa seca e liofilizada. Os valores obtidos para a atividade antioxidante pelo método ABTS$^+$ e pelo complexo de fosfomolibdênio tiveram uma diferença estatística significativa. Os dados relativos a porcentagem de proteção pelo método β-Caroteno/Ácido Linoléico demonstraram que a polpa seca não diferiu estatisticamente da polpa fresca e liofilizada. Contudo, a polpa liofilizada apresentou maior atividade antioxidante pelos ensaios ABTS$^+$ e Complexo de Fosfomolibdênio quando comparada com os outros tratamentos (polpa fresca e seca).

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INTRODUCTION

The bioactive compounds present in fruits and vegetables play important beneficial effects in human nutrition, acting mainly in the prevention of diseases related to aging and stress (FRANCINI *et al*., 2020). The most accepted definition of bioactive compounds in the literature describes them as "natural or synthetic compounds with the ability to interact with one or more components in living tissues and exert a broad biological effect" (VILAS BOAS *et al*., 2021).

Phenolic compounds are a group of phytochemicals widely distributed in fruits, vegetables, teas, olive oil, tobacco, among others. However, such substances have been reported as protective effect on health, presenting antioxidant properties, anticancer, antimutagenic, antimicrobial, anti-inflammatory, and other biological properties (GULCIN, 2020). Antioxidants, on the other hand, are natural or synthetic substances that can prevent or delay oxidative cellular damage caused by "oxidants", reducing the action of reactive oxygen species, reactive nitrogen species, and free radicals (unstable molecules or ions with unpaired electrons) (APAK *et al*., 2016). Reactive oxygen species are substances often cited as causing oxidative stress; for this reason, such substances are byproducts of aerobic metabolism that can promote the onset and development of various diseases. However, its importance in redox signaling and its role in cellular function has been strongly elucidated in the literature (JAGANJAC *et al*., 2021).

The *Litchi chinensis* Soon. popularly known as "lychee" is a fruit of Chinese origin, consisting of bioactive compounds that act in the prevention and treatment of diseases such as diabetes, hypertension, hyperglycemia, hyperlipidemia, atherosclerosis and cancer (YAO *et al*., 2021). The fruit pulp contains polyphenols, polysaccharides, vitamins, and minerals of high nutritional value, and consequently act as an adjuvant for health maintenance (ZHAO *et al*., 2021).

Given the above, fresh foods such as fruits and vegetables have high water activity and are highly sensitive to heat and degradation (ZHANG *et al*., 2017). Moreover, waste and losses of fresh foods during industrial processing have become a serious nutritional, economic, and environmental problem (SAGAR *et al*., 2018).

Thus, innovative food preservation and processing methods offer many advantages in terms of nutritional value, quality and food safety (GUZIK *et al*., 2022). Dehydration is one of the most widely used methods to increase the shelf life of foods. However, the drying methods used for food dehydration, besides being efficient and economical, should produce high-quality products based on taste, nutritional value, color, uniformity, appearance, and texture (ZHANG *et al*., 2017). In this regard, the aim of this study was to evaluate the content of total phenolic compounds and antioxidant activity of lychee pulp submitted to different dehydration methods.

MATERIAL AND METHODS

Plant material

The fruits were acquired from the local market located in Lavras-MG. The plant material was sanitized with 300 ppm sodium hypochlorite solution for 15 minutes. Afterwards, the fruits were pulped, and manually separated into peel, pulp, and seeds. The pulp was weighed, packed in plastic bags, and divided into three equal parts. The *in natura* sample was kept frozen in a -20°C freezer for further analyses. The other portion of the sample was dried in an oven with air circulation at 55±5°C until constant weight (24 hours) and a portion of the raw material was freeze-dried for 48 hours at -60°C. The temperature and time of dehydration in both methods used were determined by pre-tests considering a shorter time and a milder temperature, in order to obtain a product with lower water activity and with greater preservation of the bioactive compounds.

Extracts Preparation

The extracts were obtained according to methodology described by Samavardhana *et al*. (2015) with adaptations. An amount of 3g of *in natura*, dried and freeze-dried pulp was weighed and added to 80% ethanol. The samples were homogenized in an electronic shaker (Bioevopeak SHK-O0310III) for 30 minutes. Afterwards, the samples were transferred to the ultrasonic bath (UltraCleaner 1600A) for another 30 minutes. The extracts were filtered and stored in amber glasses

for further analysis. The extraction was performed with three repetitions for each treatment.

Total phenolic compounds

Aliquots of the extracts were transferred to microplates with 96 wells, and the Folin-Ciocalteu and Fast Blue assay were performed, according to methodology reported by Rufino *et al*. (2010) and Medina *et al*. (2011), respectively. Readings were performed in the microplate reader (EZ Read 2000, Biochrom®), at 735nm and 420nm, respectively. The results were expressed as mg gallic acid equivalents (GAE) g^{-1}. The determination was performed in triplicates.

Antioxidant capacity

The determination of the antioxidant activity by the $ABTS^{+}$ radical (2,2-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) and β-Carotene/Linoleic acid taking into account the initial (time zero) system solution was performed according to the methodology proposed by Rufino *et al*. (2010). The phosphomolybdenum complex assay was performed according to methodology described by Prieto *et al*. (1999). Aliquots of the extracts were transferred to microplates with 96 wells, and after reaction time of all the assays the readings were performed in the microplate reader (EZ Read 2000, Biochrom®), at 734nm, 470nm and 695nm, respectively. The results were expressed as % inhibition for the $ABTS^{+}$ assay, as % inhibition of oxidation for β-Carotene/Linoleic Acid assay and as mg ascorbic acid equivalent (AAE $100g^{-1}$) for the Phosphomolybdenum Complex assay. The determinations were performed in triplicates.

Water activity

Water activity (a_w) was measured in triplicate using a bench-top water activity analyzer (Series 3 TE, Aqualab®) at 25ºC.

Staining

Color was determined at five different points on each sample using a Konica Minolta CR-400 colorimeter. Measurements were taken on the CIELAB color space parameters of the Commission International de l'Éclairage: luminosity (L*) and the chromaticity coordinates (a*) and (b*) (CIE, 1996).

Statistical Analysis

Analysis of variance (ANOVA) and Tukey test for pairwise mean comparisons were performed using Statistica 10.0 software at 5% of significance level. Graphs were plotted in GraphPad Prism 9.0.2 software.

RESULTS AND DISCUSSION

The results obtained in the determination of total phenolic content in the different treatments (Table 1) ranged from 42.66 to 89.21 mg GAE g^{-1} using the Folin-Ciocalteau assay and from 6.49 to 26.50 mg GAE g^{-1} using the Fast Blue assay. In both assays the mean values of the fresh pulp were lower ($p<0.05$) when compared to the dried and freeze-dried methods. According to the study conducted by Lal *et al.* (2023), fresh lychee pulps were analyzed for their total phenolic compound content (Folin-Ciocalteau), however, the results were lower (2.23 (2018) to 2.88 (2019) mg GAE g^{-1}) than those obtained in the present study. Phenolics contents can vary according to geographical origin, plant variety, climate, soil type, planting region, agronomic and environmental factors (Mykhailenko *et al.,* 2022), which may justify the disparity between the results.

In the determination of phenolic compounds, the Folin-Ciocalteau assay showed superior results when compared to the Fast Blue assay. Due to this fact, the Folin-Ciocalteau method may suffer interferences due to the presence of non-phenolic antioxidant constituents and reducing substances, such as ascorbic acid, glucose, fructose and sulfites, besides some amino acids and proteins, affecting the final results (BARROS *et al*., 2020). On the other hand, the method developed by Medina (2011), called Fast Blue, uses the Fast Blue diazonium salt, in which the diazonium group reacts with reactive hydroxyl groups of the phenolic compounds, forming stable azo complexes. Thus, in the Fast Blue assay, the results suffer less interference and, consequently, lower values are obtained (MEDINA, 2011).

Table 1. Mean values ± standard deviations of total phenolic compounds and antioxidant activity of *Litchi chinensis* pulp

Assay	*In natura*	Dried	Freezedried
Folin-Ciocalteau (mg GAE g^{-1})	42.66±3.64^{b}	89.21±6.38^{a}	84.51±5.65^{a}
Fast Blue (mg GAE g^{-1})	6.49±0.63^{b}	26.50±4.17^{a}	25.26±1.38^{a}
$ABTS^{+}$ (% Inhibition)	17.21±1.70^{c}	47.20±7.41^{b}	54.25±5.92^{a}
β-Carotene/Linoleic Acid (% Inhibition)	30.63±0.26^{b}	27.36±0.26^{ab}	31.94±2.53^{a}
Phosphomolybdenum Complex (AEE 100 g^{-1})	16.74±0.97^{c}	46.05±3.22^{b}	51.12±3.27^{a}

Means followed by the same letters in the row do not differ significantly between the treatments by the Tukey test ($p>0.05$)

The lychee pulp, regardless of the dehydration method applied, presents phenolic compounds that are essential for health maintenance.

In the determination of antioxidant activity by the $ABTS^{+}$ method (Table 1) it was possible to observe statistically significant difference ($p<0.05$) when compared to the different treatments. The percentage of protection of the fresh pulp was only 17.21%±1.70% compared to the freeze-dried pulp (54.25%±5.92%). The study performed by An *et al.* (2022) compared various methods of drying for lychee pulp, in which the freeze-dried pulp showed higher antioxidant activity by the $ABTS^{+}$ method. According to Santos and Silva (2020), $ABTS^{+}$ is a stable blue-green chromophore cation radical, which loses its color in the presence of an antioxidant molecule. The $ABTS^{+}$ radical is formed by the reaction of a strong antioxidant agent (potassium permanganate or potassium persulfate) together with the $ABTS^{+}$ salt. The interpretation of the result obtained is simple, since the higher the value obtained, the higher the antioxidant activity of the sample.

The data regarding the percentage of total phenolics protection obtained using the β-Carotene/Linoleic Acid method showed that the dried pulp did not differ statistically from the fresh and freeze-dried pulp. This result suggests that the drying process did not interfere in the antioxidant compounds that protect the β-Carotene from suffering discoloration by the presence of oxidative degradation products of linoleic acid. In this regard, the β-Carotene/Linoleic Acid assay consists of the basic principle that linoleic acid (unsaturated fatty acid) will be oxidized by the reactive oxygen species generated in oxygenated water (GULCIN, 2020). The antioxidant activity of this method can be classified as high (>70% oxidation inhibition), intermediate (between 40% and 70%), and low (<40%) (RUFINO *et al*. 2010). Thus, the antioxidant activity can be classified as low for all treatments. The antioxidant activity of the different treatments for 120 minutes is shown in Figure 1.

Based on the results from Figure 1, we can observe another aspect of the β-Carotene/Linoleic Acid test, where we can follow the oxidation process of the samples for 120 minutes.

In the determination of antioxidant activity by the phosphomolybdenum complex method, a statistically significant difference ($p<0.05$) was observed among the samples (Table 1). The freeze-dried pulp presented higher antioxidant activity values (51.12%±3.27%), and again the fresh pulp presented the lowest results (16.74%±0.97%). The technique applied in the phosphomolybdenum complex assay is based on the reduction of Mo^{6+} to Mo^{5+} by the sample analyte and subsequent formation of a Mo^{5+} green phosphate complex at acidic pH (GULCIN, 2020). Considering that the assay uses ascorbic acid as a standard, it can be observed that the higher the ascorbic acid equivalence value obtained, the higher the antioxidant activity of the sample. Therefore, the antioxidants present in the extract were able to provide the reduction of Mo^{6+}.

The foods can be classified according to their a_w into three groups: foods with low a_w (up to 0.60), intermediate a_w (between 0.60 and 0.90) and high a_w (greater than 0.90).

Thus, as shown in Figure 2, the lychee pulp *in natura* has high water activity, the freeze-dried

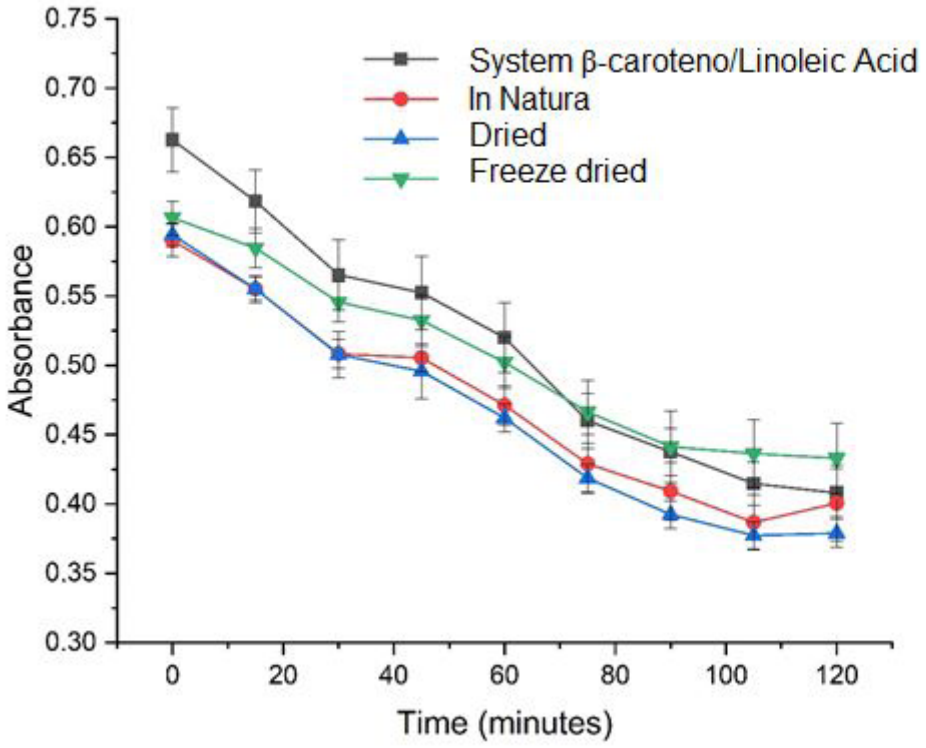

Figure 1. Antioxidant activity evaluated by the β-Carotene/Linoleic Acid complex method during 120 minutes of degradation

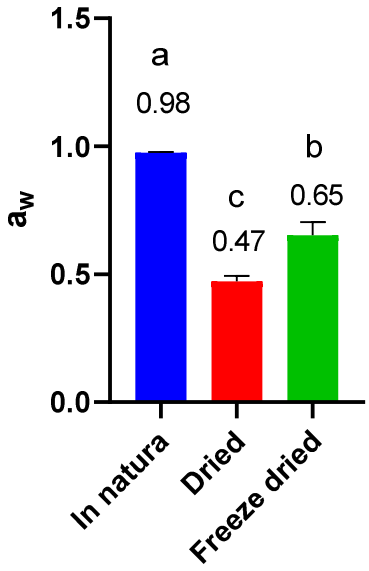

Figure 2. Water activity of fresh, dried and freeze-dried pulp

pulp has intermediate water activity, and the dried pulp has low water activity. In the water activity range of fresh lychee pulp, dilute solutions may occur with food components, providing substrates for the growth of microorganisms, while enzymatic reactions and lipid oxidation may have their speed reduced (RIBEIRO; SERAVALLI, 2007).

Fruits and vegetables are products with high perishability, mainly because they contain water content higher than 80%. Thus, drying or dehydration is an essential process for the conservation of fruits, vegetables and grains, since the reduction of water activity in the drying process increases the storage stability of food (PANDISELVAM *et al.*, 2021). Given the above, it is evident that the results presented for the water

activity of the dried and freeze-dried pulp were effective in reducing the moisture content, and consequently, increased the shelf life of the food.

In Table 2 is presented the results obtained in relation to the color parameters of the lychee pulp.

Table 2. Mean values ± standard deviations of color parameters L*, a* and b* of *Litchi chinensis* pulp

	In natura	**Dry**	**Freeze-dried**
L*	55.89±2.47[b]	58.47±0.78[b]	74.83±1.64[a]
a*	-3.20±0.47[b]	5.71±0.28[a]	-1.62±0.43[c]
b*	4.88±0.52[b]	21.30±0.12[a]	19.53±1.95[a]

Means followed by different letters in the row differ significantly between the treatments (Tukey $p<0.05$). In this color system, L* represents the luminosity (L=0 - black and L=100 - white), a* and b* are the color coordinates responsible for chromaticity: (+a*= red and -a*= green, +b*= yellow and -b*= blue)

The L values differed statistically among the samples, being a parameter that evaluates the sample's capacity to reflect or transmit light. Thus, the values presented show that the freeze-dried pulp obtained higher values of L*, tending more towards white coloration. In relation to the a* parameter, it was possible to observe that the samples differed statistically among themselves, with the dried pulp tending to red coloration (+a*), which in fact, when exposed to drying temperature, darkening reactions occur. The b* values did not differ between the dried and freeze-dried samples.

According to the results presented, freeze-drying was the method that obtained the highest antioxidant capacity by $ABTS^+$ radical and phosphomolybdenum complex assay (Table 1), even though the analyses of total phenolic compounds did not show significant differences between dried and freeze-dried treatments. In addition, the water activity of the freeze-dried pulp was intermediate, suggesting that this dehydration method can increase the shelf life of foods. It is important to highlight that each methodology evaluates different aspects related to the mechanism of antioxidant action of the sample. Thus, it is suggested that oven drying was possibly able to degrade, in a small amount, phenolic compounds that were in low concentration and that were responsible for the antioxidant action evaluated only by the methodologies that differed statistically. Although losses in vitamins and other bioactive compounds occur, freeze-drying is the best dehydration method when compared to other methods, mainly because it preserves the nutritional attributes of foods (BHATTA *et al*., 2020).

The use of temperature during the drying process leads to adverse effects on the food, including hardening on the surface and poor quality attributes (PHAM *et al.,* 2017). However, recent studies have investigated other drying techniques with special attention to industrial food applications, elucidating methods that can preserve phytoconstituents characteristics, nutrients, and storage stability (FATHI *et al.,* 2022).

During the drying process, excessive water transport results in many physical changes that compromise food quality, such as changes in raw material volume and in texture, and shrinkage of the product (PHAM *et al.,* 2017). Although drying has some disadvantages, it is a more economically viable method, since freeze-drying requires long raw material processing times, high operating cost, and energy consumption (BHATTA *et al.,* 2020).

Based on the previous information, it is possible to state that freeze-drying was the best drying method, especially regarding the preservation of the antioxidant potential and shelf-life of the pulp. However, for commercial purposes, it is necessary to conduct an economic viability study to evaluate whether the costs related to the process would make obtaining the product unfeasible.

CONCLUSION

- The different treatments presented, in their composition, contents of total phenolic compounds with antioxidant activity. However, it was observed that the freeze-dried pulp obtained the highest antioxidant potential by the $ABTS^+$ radical and phosphomolybdenum complex assays when compared to the dried pulp. Further studies should be conducted to evaluate the sensory acceptance and economic viability of the pulps.

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AUTHORSHIP CONTRIBUTION STATEMENT

OLIVEIRA, J.P.L.: Conceptualization, Investigation, Methodology, Writing – original draft; **SANTOS, I.A.:** Conceptualization, Data curation, Investigation, Methodology, Writing – original draft; **SOUZA, L.R.:** Conceptualization, Data curation, Methodology, Writing – original draft; **CÂNDIDO, G.S.:** Data curation, Formal Analysis, Investigation, Writing – review & editing; **FRANCO, M.:** Conceptualization, Investigation, Software, Writing – original draft, Writing – review & editing; **VILAS BOAS, E.V.B.:** Project administration, Resources, Software, Writing – original draft, Writing – review & editing; **CARVALHO, E.E.N.:** Conceptualization, Project administration, Software, Writing – original draft, Writing – review & editing.

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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