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Bioactive phytochemicals and antioxidant activity in fresh and dried lychee fractions[1]

Fitoquímicos bioativos e atividade antioxidante de frações de lichia frescas e secas

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ABSTRACT - Fruit of the lychee cv. Bengal are approximately 50% peel and seeds, which are discarded. These by-products have antioxidant compounds which are capable of blocking the harmful effects of free radicals in the body. Bioactive compounds (ascorbic acid, beta-carotene, lycopene and phenols) and antioxidant activity were evaluated in different extracts, both fresh and dried at 45 °C, of the skin, pulp and seeds of the lychee, which were subjected to principal component analysis to clarify which of the compounds are responsible for this activity. Principal component analysis explained 82.90% of the variance of the antioxidant profile of the lychee. The peel displayed higher levels of phenols, ascorbic acid, beta-carotene and antioxidant activity, while the seeds stood out due to their levels of lycopene. With drying, there was a decrease in the levels of ascorbic acid and beta-carotene and in antioxidant activity, with an increase in the levels of phenols and lycopene. The antioxidant activity found in the peel and seeds of the lychee is high, and is mainly due to ascorbic acid and beta-carotene, as demonstrated by principal component analysis, allowing the use of these fractions as sources of natural antioxidants.

Key words: Lychee. Antioxidants**.** Principal component analysis.

RESUMO - Os frutos da lichieira cv. Bengal possuem aproximadamente 50% de casca e semente, que são descartadas. Estes subprodutos apresentam compostos antioxidantes capazes de bloquear os efeitos danosos dos radicais livres, no organismo. Foram avaliados os compostos bioativos (ácido ascórbico, betacaroteno, licopeno e fenóis) e a atividade antioxidante, em diferentes extratos da casca, polpa e semente de lichia, *in natura* e secas a 45 ºC, os quais foram submetidos à análise de componentes principais, para elucidar quais compostos são responsáveis por esta atividade. A análise de componentes principais explicou 82,90% da variância do perfil antioxidante da lichia. A casca apresentou maiores teores de fenólicos, ácido ascórbico, betacaroteno e atividade antioxidante, enquanto a semente destacou-se pelos teores de licopeno. Com a secagem, houve decréscimo nos teores de ácido ascórbico e betacaroteno e na atividade antioxidante e aumento nos teores de fenólicos e licopeno. A atividade antioxidante encontrada na casca e semente de lichia é elevada e se deve, sobretudo, ao ácido ascórbico e ao betacaroteno, conforme demonstrado pela análise de componentes principais, o que possibilita o uso destas frações como fontes de antioxidantes naturais.

Palavras-chave: Lichia. Antioxidantes. Análise de componentes principais.

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INTRODUCTION

The lychee (*Litchi chinensis* Sonn.), a species of the *Sapindaceae* family suited to subtropical-climates, is of Chinese origin and is perfectly adapted to Brazilian climatic conditions. Fruit of the lychee cv. Bengal are about 50% peel and seed, which are discarded by both industry and consumers (KUMAR; KUMAR; SHARMA, 2012). Lychee production in the country has still not been determined (SMARSI *et al*., 2011), but is focused mainly in the southeast. In Brazil, industries that benefit from the lychee use the pulp in the preparation of ices and jams.

Recent work has been carried out, aiming to evaluate the nutritional potential of the by-products of lychee fruit, with initial results indicating that the peel and seed have high energy and nutritional potential (QUEIROZ; ABREU; OLIVEIRA, 2012). In addition, the peel and seed are rich in antioxidants such as ascorbic acid, phenolic compounds including gallic acid, flavonoids (procyanidin B4, procyanidin B2and epicatechin), and anthocyanins (cyaniding 3-rutinoside, cyanidin-3-glucoside, quercetin 3-rutinoside and quercetin 3-glucoside). Pharmacological studies indicate that the by-products of the lychee have various effects including anti-inflammatory, anti-hyperlipidemic, anti-hyperglycemic, hepatic and cardioprotective, as well as having high antioxidant activity (BHOOPAT *et al*., 2011; JIANG *et al*., 2013; XU *et al*., 2011).

A wide variety of *in vivo* and *in vitro* methodologies have been used to verify the antioxidant activity of isolated substances in food or drink. There is no one universal method by which this activity may be measured, since all methods have both advantages and disadvantages. More than one are therefore employed to determine this activity, such as the DPPH$^{\cdot}$ method (2.2-diphenyl-1-picrylhydrazyl) based on the capture of the DPPH$^{\cdot}$ radical by antioxidants (SÁNCHEZ-MORENO, 2002), and the beta-carotene/ linoleic acid system, based on the oxidation of beta-carotene, induced by the products of the oxidative degradation of linoleic acid (DUARTE-ALMEIDA *et al*., 2006).

The fruit, recognized as a source of vitamins, minerals and fibre, is nutritionally important to our diet. In recent years, greater attention has been given to these foods, since evidence suggests that regular consumption of fruit is associated with reduced mortality and the morbidity of some chronic diseases (BHOOPAT *et al*., 2011; DEMBITSKY *et al*., 2011), as they contain in addition to nutrients, bioactive substances such as vitamins and secondary metabolites, which are capable of carrying out pharmacological activities.

Recent clinical and epidemiological studies show that phytochemicals are the major bioactive compounds of fruit, and prove that their consumption helps reduce the risk of cardiovascular diseases and cerebrovascular accidents, as well as reducing the incidence of certain types of cancers and oxidative stress (BHOOPAT *et al*., 2011; DEMBITSKY *et al*., 2011).

Given this advantage, the bioactive compounds and total antioxidant activity were evaluated in different extracts of the peel, pulp and seed of lychee fruit *in natura*, and in the peel and seed after being dried at 45 °C.

MATERIAL AND METHODS

Fruits of the lychee cv. Bengal from the 2010/2011 crop were harvested in a commercial orchard in Nepomuceno, in the Brazilian state of Minas Gerais (MG), (21º20 'S, 45º23' W), and transported to the Biochemistry Laboratory of the Department of Chemistry at the Federal University of Lavras. They were selected for uniform colouration of the peel (intense red), for their medium size and absence of defects. They were washed, sanitised with 200 μL L^{-1} sodium dichloroisocyanurate for 15 minutes, weighed, divided into two lots of 140 fruits and separated into peel, pulp and seed.

The peel, pulp and seed from the first batch were frozen in liquid nitrogen and stored in a freezer (-20 °C) until the analyses were carried out. The peel and seed from the second batch were dried in an oven at 45 °C to constant weight, and stored in amber flasks; four days being needed to dry the peel and eight days to dry the seed.

The phenolic compounds were extracted with 50% methanol and quantified by the Folin-Denis method as described by Association of Official Analytical Chemistry (2012). The results were expressed in mg of phenolic compounds (equivalent to tannic acid) 100 g^{-1} of dry matter (DM).

The ascorbic acid was extracted in 0.5% oxalic acid with 0.1 g of added diatomaceous earth, quantified according to the methodology recommended by Strohecker and Henning (1967), and expressed in mg of ascorbic acid 100 g^{-1} DM.

The levels of beta-carotene and lycopene were determined according to Nagata and Yamashita (1992). To do this they were extracted with a solution of acetone and hexane (4:6) (v/v) in an ultrasonic bath at 4 °C for 5 minutes. After being filtered, the absorbances of the extracts were read in the 453 nm, 505 nm, 645 nm and 663 nm wavelengths.

To evaluate the antioxidant activity, four extracts were prepared using as the extracting solutions, distilled water (A), acetone (B), a 1:1 (v/v) 70% acetone and 50% ethanol solution (C) and 50% methanol (D). The first three extracts (A, B and C) were obtained by agitating the samples in their respective solutions for 2 hours. The fourth extract (D) was obtained by extraction with 50% methanol in a reflux condenser at 80°C; the methanol being removed by evaporation and the volume of the extract increased with distilled water.

Antioxidant activity was determined by the DPPH· method, following Sánchez-Moreno (2002) with modifications. Three dilutions of the extracts A, B, C and D were prepared. To 0.1 mL of each dilution was added 3.9 mL of 0.06 mM DPPH· solution with the corresponding solvent being used as a blank. The absorbance at 515 nm was determined at the start and again after 30 minutes, the time for the absorbance to stabilise. The results were expressed in percentage of antioxidant activity.

Antioxidant activity by the carotene/linoleic acid system was determined according to Duarte-Almeida *et al*. (2006), with modifications. Three dilutions were prepared from each of the extracts A, B, C and D, and 0.2 mL of each dilution were mixed with 2.5 mL of the carotene/linoleic acid system solution. As control, 0.2 mL of a trolox in ethanol solution (200 mg L^{-1}) was used with 2.5 mL of the system solution. The tubes were kept in a water bath at 40 °C for 2 hours. A reading (λ = 470 nm) was taken 2 minutes after preparing the reaction mixture, and every 30 minutes for 2 hours. The results were expressed in percentage of antioxidant activity.

The experiment was carried out in a completely randomised design (CRD), consisting of 5 treatments (peel, pulp and seed *in natura*, and pulp and seed dried at 45 °C), using seven replications of 20 fruits. The results underwent variance analysis using the Sisvar statistical software (FERREIRA, 2011), and the treatment means were compared by Tukey test at 5% probability. Additionally, the Octavie 3.4.3 software (EATON, 2013) was used on the results to determine the principal components, and for this the data were centred around the mean and auto-scaled.

RESULTS AND DISCUSION

There was a significant difference (>0.05) between the dried fractions and those *in natura* for all the parameters under test. The moisture content of the peel, pulp and seed were 68.93, 83.91 and 47.11 g 100 g^{-1} respectively, with the highest water content being noted in the pulp.

Between the fractions *in natura*, the seed showed the lowest content for phenolic compounds (11.45 mg 100 g^{-1} DM), with no significant differences being found ($p<0.05$) between contents for the peel and pulp (22.04 and 21.20 mg 100 g^{-1} DM respectively), while the dry fractions showed levels for phenolic compounds which were significantly higher than those *in natura* ($p>0.05$) (Table 1), especially for the peel. Just as observed in grape and jabuticaba, these compounds are present in higher concentrations in the peel and seed (BOARI LIMA *et al*., 2008).

The higher content found for phenolic compounds in the dried peel and seed can be explained: it is believed that phenolic compounds in fruits are in the free form and/or associated with polysaccharides of the cell wall through hydrogen bonds between the hydroxyl groups of the phenolic compounds and the oxygen atoms of the polysaccharides (PINELO; ARNOUS; MEYER, 2006), and with drying there is a change in the nutritional value and in the physical and structural properties of fruits and vegetables. Even when this occurs at mild temperatures (50 °C for orange peel, 45°C for grape and jabuticaba peel), polymers in the cell wall are destroyed, particularly pectic substances, but in lesser amounts than at high temperatures (EBUN; SANTOSH, 2011; HARBOURNE *et al*., 2009; KIM *et al*., 2006). Removal of water from the lychee fractions may therefore have caused degradation of the cell wall, with the consequent hydrolysis releasing linked phenolic compounds and making them soluble, facilitating their extraction and resulting in an increase in the levels of the compounds seen in the dried peel and seed in relation to the fractions *in natura*.

Studies show that heating at temperatures of between 50 and 150 °C can convert insoluble phenolic compounds into soluble phenolics in grape peel and in broccoli, potatoes and onions, but it is unable to achieve this conversion in carrots and in cabbage cooked in the same way, or in rice hulls which have been dried at 100 °C (FALLER; FIALHO, 2009; KIM *et al*., 2006; LEE *et al*., 2003); this

Table 1 - Phenolic compounds (mg 100 g^{-1} DM), ascorbic acid (mg 100 g^{-1} DM), beta-carotene (mg 100 mL^{-1}) and lycopene (mg 100 mL^{-1}) for fractions of lychee fruit, Lavras, MG, 2011

Fraction	Phenolic compounds[(1)]	Ascorbic acid	Beta-carotene	Lycopene
Peel in natura	22.04 C	2.169.52 A	261.99 A	- (2)
Seed in natura	11.45 D	370.47 C	0.07 D	0.12 B
Pulp in natura	21.20 C	453.19 B	36.24 C	-
Dried peel	71.71 A	225.98 D	195.09 B	-
Dried seed	34.72 B	75.67 E	2.70 D	4.33 A

Means followed by the same letter in a column do not differ by Tukey test at 5% probability; [(1)] Expressed in equivalents of tannic acid; [(2)] Not detected

indicates that the phenolic compounds in plants have linkages which are distinct. The effect of temperature on the release of phenolic compounds in different species therefore may not be the same (KIM *et al*., 2006).

The peel *in natura* displayed the highest levels of ascorbic acid (2,169.52 mg 100 g^{-1} DM), while for the other fractions, fresh or dried, the levels varied between 75.67 and 453.19 mg 100 g^{-1} ascorbic acid. Drying significantly influenced these levels, with a decrease being seen in ascorbic acid levels after the process. This is because ascorbic acid is susceptible to degradation by temperature, pH, oxygen and the enzymes which influence the degradative mechanisms, altering the concentration of the ascorbic acid, and the ratio of ascorbic acid to dehydroascorbic acid (MAEDA *et al*., 2007).

Although there were losses of ascorbic acid, the dried fractions presented relatively high levels (225.98 and 75.67 mg ascorbic acid 100 g^{-1} DM, for the dried peel and seed respectively), especially for the dried peel, where the content was equivalent to 70.21 mg 100 g^{-1} fresh matter (FM) and was higher than that found for jabuticaba or pineapple (19.67 and 17.65 mg ascorbic acid 100 g^{-1} FM respectively) (OLIVEIRA *et al*., 2003; REINHARDT *et al*., 2004). The by-products of the lychee are therefore seen to be sources of ascorbic acid, and may be used for enriching foods, even after being dried.

The highest levels of beta-carotene were found in the lychee peel, especially in the peel *in natura*, which had a level which was superior to that of the other fractions (261.99 mg 100 mL^{-1}). Drying significantly reduced the levels of beta-carotene in the peel, since processing is the main factor influencing degradation of the carotenoid. Thus the loss of tissue integrity, the contact with oxygen, the light and the rise in temperature during processing may all have caused degradation (PÉNICAUD *et al*., 2011). This behaviour was not seen in the seed however, where carotene content for these fractions, both dried and *in natura*, were not significantly different ($p<0.05$).

The beta-carotene is the main source of vitamin A, so even with losses due to processing, results show the nutritional potential of lychee peel as a source of beta-carotene, possibly contributing to the enrichment of diets and food products despite the bioavailability not having been studied.

The lycopene content in the seed was 0.12 mg 100 mL^{-1}, the carotenoid not being detected in the other fractions. With drying, there was an increase in the lycopene content of the seed, a behaviour which differs from that observed by Zanoni *et al*. (1999), who found a 12% loss in lycopene levels for tomatoes dried at 110 °C, however no losses in levels of the nutrient were detected at 80 °C.

Antioxidant activity based on inhibition of the DPPH$^{\cdot}$ radical was high for all the extracts under analysis, ranging from 12.77 to 87.18% (Table 2). Noteworthy is extract D, prepared with 50% methanol followed by evaporation, which presented a high sequestration capacity for the DPPH• radical, displaying the greatest percentages for antioxidant activity.

Methanolic and ethanolic solutions are efficient in extracting phenolic compounds, particularly the more polar compounds such as flavonols, which indicates that the high antioxidant activity observed for this extract may be associated with the presence of these compounds or of other extractable compounds, such as anthocyanins derived from hydroxycinnamic and ellagic acids, ascorbic acid and small amounts of carotenoids, which would increase the AA. (KAJDŽANOSKA; PETRESKA; STEFOVA, 2011; MATINEZ-VALVERDE; PERIAGO; PROVAN, 2002).

The percentage of antioxidant activity observed in lychee peel for extract D, was 87.18%, higher than that found by Prasad *et al*. (2009), who evaluated the antioxidant activity in the peel of the lychee cv. Baila subjected to extraction at high pressure and found 74% activity, and by Duan *et al*. (2011) who found antioxidant activity in the lychee cv. Huaizhi ranging from 60 to 25% during storage, using a methanolic solution as extractant. The differences seen between the antioxidant activity of

Table 2 - Percentages of antioxidant activity of extracts A (aqueous), B (acetone), C (1:1 solution, 70% acetone and 50% ethanol) and D (50% methanol) in fractions of lychee fruit, for the DPPH$^{\cdot}$ radical, Lavras, MG, 2011

Fraction	A	B	C	D
Peel in natura	25.70 C	63.64 A	56.58 A	87.18 A
Seed in natura	47.92 A	27.67 B	39.12 C	78.42 B
Pulp in natura	41.95 B	27.25 B	42.36 B	31.71 D
Dried peel	16.73 D	19.60 C	21.38 E	40.55 C
Dried seed	15.42 D	12.77 D	32.79 D	18.13 E

Means followed by the same letter in a column do not differ by Tukey test at 5% probability

the fruits in this study and in that of the above authors are due to the different types of climate, maturity, cultivation and agricultural practices.

The percentage of antioxidant activity by the beta-carotene/linoleic acid system varied between 21.63 and 75.89%, with the highest antioxidant activity being seen in extract B for the peel *in natura*, as shown in Table 3.

In all the extracts under analysis, the peel *in natura* showed the greatest antioxidant activity by this method, however variable results are observed when compared to those seen with DPPH• sequestration, with lower percentages for antioxidant activity, and the extracts with the greatest antioxidant activity being different for each test. Kubola and Siriamornpun (2008) evaluated the antioxidant activity of different parts of *Momordica charantioa* L. and found different results for each method being analysed, demonstrating that one sample may carry out different activities in different tests.

For all the methods being analysed, there was a decrease in antioxidant activity with drying, indicating a loss of potentially antioxidant compounds. The behaviour of antioxidant activity by the DPPH• method is similar to that observed with ascorbic acid, from which it can be inferred that one of the factors responsible for the loss of antioxidant activity is the degradation of ascorbic acid due to time and exposure to oxygen during the drying process.

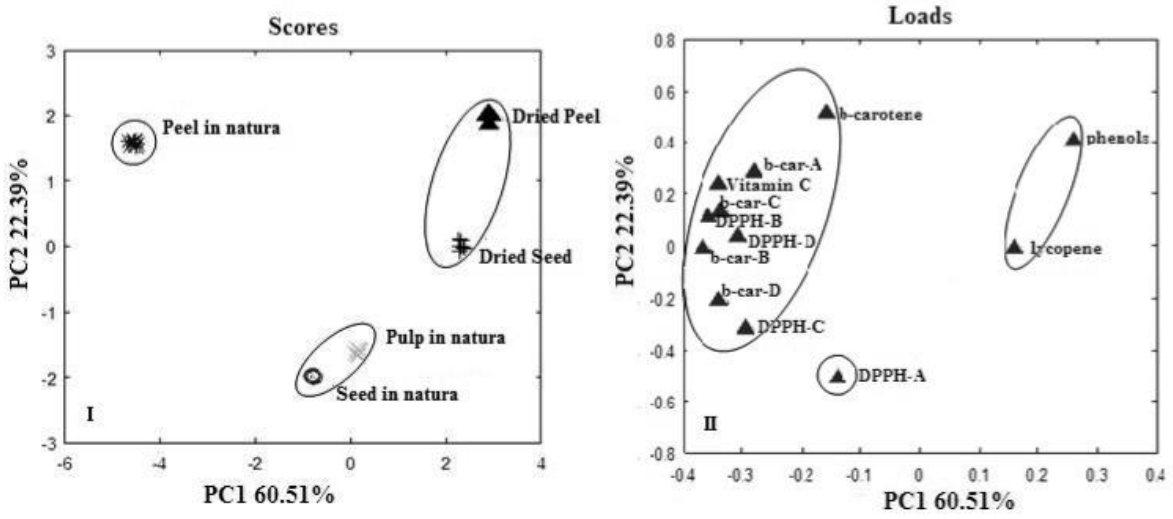
Principal component analysis (PCA) applied to the data explained 82.90% of the total variance in the antioxidant profile of the lychee (Figure 1). The first component (PC1) explains 60.51% of all information, and separates horizontally

Table 3 - Percentage of antioxidant activity of extracts A (aqueous), B (acetone) C (1:1 solution, 70% acetone and 50% ethanol) and D (50% methanol) in fractions of lychee fruit, at a concentration of 1 mg mL^{-1}, by the beta carotene/linoleic acid system, Lavras, MG, 2011

Fraction	A	B	C	D
Peel in natura	66.11 A	75.89 A	75.35 A	75.47 A
Seed in natura	22.66 D	41.44 C	45.82 B	61.22 B
Pulp in natura	24.94 C	56.44 B	36.37 D	62.12 B
Dried peel	21.63 E	28.53 D	28.82 E	29.53 D
Dried seed	36.86 B	28.51 D	43.43 C	47.14 C

Means followed by the same letter in a column do not differ by Tukey test at 5% probability

Figure 1 - Graphical representation of scores (I) and loads (II) from analyses of fractions of lychee fruit, evaluated in relation to the axes defined by the principal components (CP1 and CP2). In II, (b-car) corresponds to AA by the beta-carotene/linoleic acid system and (DPPH) to AA for the sequestration of the DPPH radical, for extracts of fractions of lychee fruit prepared with: (A) water, (B) acetone, (C) 70% acetone/50% ethanol (v/v), (D) 50% methanol



the fractions which have the highest levels of phenolic compounds (dried peel and seed) (Figure 1I). The second component (PC2) discriminates vertically the peel *in natura* from the other fractions, by the levels of beta-carotene, ascorbic acid and antioxidants, explaining 22.39% of the total variance. There is separation of the lychee fractions into three groups, and it can be seen that the peel *in natura*, which has more antioxidant activity, is distanced from the remaining fractions. Along the CP1 axis, there is separation of the dried fractions and those *in natura*, which reflects the type of treatment applied to the lychee fractions.

The loads characterise the trends between variables (Figure 1 II). Along the CP1 axis, it can be seen that the variables which most influenced this component are: (horizontally) phenols and lycopene, respectively the main variables for the dried peel and dried seeds, and ascorbic acid and antioxidants, the main variables for the peel *in natura*; and (vertically) the levels of beta-carotene (with positive values) and the antioxidant activity of extract A by the DPPH method (with negative values). It can also be seen that the antioxidant activity found in the lychee fractions *in natura*, is due to the ascorbic acid and beta-carotene, a result that can be deduced by their proximity to the antioxidant extracts.

CONCLUSIONS

1. The lychee fractions showed high antioxidant activity, especially the peel *in natura*;
2. The antioxidant activity seen in the peel *in natura* is due to the levels of ascorbic acid and beta-carotene found in this fraction;
3. Drying at 45 °C reduces the antioxidant activity and the levels of ascorbic acid in the peel and seed of the lychee;
4. Even with the losses in antioxidant activity from drying, the peel and seed present significant antioxidant activity, which allows the use of by-products of the lychee as sources of natural antioxidants.

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