OBTAINING ANTIOXIDANT COMPOUNDS SEED *TAMARINDUS INDICA*, SWEET VARIETY

Páulia Maria Cardoso Lima Reis*, Haiko Hense[1], Claudio Dariva[2], Elton Franceschi[2], Gizelle Ângela Barroso Vieira[3]

*Masters student in Engineering in Food by the UFSC
[1]Advisor Teacher of Graduate Course of food engineering at UFSC
[2]Teachers Co-advisors Tiradentes University
[3]Teacher Co-advisor at IF Sertão-PE
Universidade Federal de Santa Catarina
Campus Universitário, Florianópolis, Brasil

Email: pauliamaria@pop.com.br

Abstract. The seeds of *Tamarindus indica*, sweet variety, are discarded without use of technology. The natural antioxidants can be found in in vegetables raw materials, therefore, the objective of this study was to obtain the seed extract and evaluation of their antioxidant activity (AA). The experiment was conducted using the linear outlining 2^2 + 3 repetitions at the center point, having CO_2 as solvent, at pressures 100, 175 and 250 bar and temperatures 68, 95 and 122 F. In the condition of 250 bar and 122 F was used CO_2 + ethanol at concentrations of 10, 25, 50 and 75%. The AA was assessed by the methods of DPPH and Folin-Ciocaltou. It was observed that by increasing the density, the higher the yield of extraction with pure CO_2 due to the greater solvating power of the solvent, obtaining 2,6% 250bar/122 F. The increasing concentration of ethanol, 10 to 75%, contributed to a yield, respectively, from 3,47 to 20,3%, for it increased, the solubility of polar compounds. Increasing pressure and temperature favored the AA with pure CO_2 from 28,2 to 53,9% (500μg/mL), but also the increasing ethanol concentration increased from 25,1 to 97,8% (500μg/mL). Phenolic compounds extracted with pure CO_2 showed linear variation of 6,13 to 25,1 mgEAG/g extract, on condition of 100bar/68 F and 250bar/122 F, respectively. The ethanol contributed significantly to AA, being found of 25,6 to 420,8 mgEAG/g extract, 10 and 75%, respectively, this can be attributed to its chemical similarity to these compounds. The seed of the tamarind candy is potentially promising as a source of natural antioxidants.

Keywords: antioxidants, extraction, seed, tamarind.

1. Introduction

The tamarind (*Tamarindus indica* L.) is a fruit plant that belongs to the family of legumes, native to equatorial Africa, India and Southeast Asia and grows in tropical and subtropical regions, with an average temperature of 25 ºC. Ideal It is considered an ideal tree for semiarid regions, tolerating 5-6 months of drought conditions, but can not survive in cold temperatures. There are several varieties of *Tamarindus indica*, they may be divided into acidic and sweets. The acidic variety is commonly found in most countries because it develops easily in hot and sunny [1].

Luengthanaphol et. al. [2] (2004), reported that two species of the tamarind are found in abundance in Thailand, where they are consumed in the form of pulp and jellies. The tamarind pulp is used in semi-arid region of Brazil, to produce juice, candy, ice cream, liquors and pastes, their seeds may be used as juices stabilizers. The seeds, however, are considered waste, discarded without any technological advantage on most small producing farms in the region of the São Francisco Valley.

According to Ordinance nº. 540 - SVS / MS [4] of 27 October 1997, antioxidants are substances that delay the appearing of oxidative modification in food. Can also be defined as additives able to prevent the harmful effects of oxidation.

Antioxidants are important to the health of human beings, because they attack free radicals that cause damage to our body. Free radicals are chemical species that contain one or more unpaired electrons, a characteristic that confers instability, short half-life and high chemical reactivity. This high chemical reactivity is largely responsible for the instability of this species, because these molecules tend to react with another compound that is present in structures close to its formation, passing to behave, thus as receivers (oxidizing) or as donors (reducing) electrons [5].

Phenolic compounds and flavonoids are often found in fruits and vegetables. Melo et al. [6] (2008) found significant quantities of phenolic compounds in 15 fruits marketed in the downtown of Recife-PE/Brasil. Several studies have shown the antioxidant potential of fruit, vegetables, seeds, fruit peel, drinks and so on.

The food industry uses synthetic antioxidants to inhibit oxidative processes in lipid food. However, there is a demand for studies on extraction and commercial viability of natural antioxidants, since the synthetic antioxidants has been the subject of questions regarding their safety [7].

Peres et al. [8] (2011), observed antioxidant activity by DPPH (diphenyl-picrylhydrazyl) in the *tamarindus indica* peels and in the inactivity to its pulp. Tsuda et al. [9] (1994) studied the extraction of antioxidants from Indian Tamarind and found four antioxidants in the seed coat (-)-epicatechin, ethyl 3,4-diidroxifenila, 3,4-diidroxibenzoato methyl and 2-hydroxy- 3,4-diidroxiacetofenona. Siddhuraju [10] (2007), report the presence of antioxidant activity (64,5 to 71,1%) in the seed of *Tamarindus indica* L. Luzia and Jorge [11] (2010), found 49,30 mg GAE / $g_{extrato}$ phenolic compounds in ethanolic solution of *Tamarindus indica* L. seed, while Simas et al. [12] (2011), found 183,28 mg GAE / mg of extract in the said fruit peels.

The aim of this work was to obtain the seed extract of *Tamarindus indica*, sweet variety of backlands of Bahia, in different conditions of temperature and pressure and evaluation of antioxidant activity.

2. Materials and methods

The raw material was collected at Bebedouro Farm in the city of Sento Sé - BA / Brazil, in the months of December 2011 and January 2012, harvest season. The seeds of the sweet tamarind were dried at 104 F for 24 hours and milled, being classified on 20-80 mesh sieves. They were then stored under 24,8 F refrigeration.

The subcritical and supercritical fluid extractions were performed with pure CO_2 through experiment delimitation 2^2 + 3 replicates at the center point, according to Table 1:

Table 1. Factorial design matrix 2^2 + 3 central points

Experiments	Temperature/F	Pressure/bar
1	68	100
2	122	100
3	68	250
4	122	250
5	95	175
6	95	175
7	95	175

And the extractions with CO_2 combined with ethyl alcohol in different concentrations occurred at 250 bar and 122 F according to Table 2:

Table 2. Planning extraction matrix with CO_2 + ethyl alcohol

Experiments	Ethanol concentration
1	10%
2	25%
3	50%
4	75%

The supercritical extraction equipment consists of a CO_2 cylinder with 99.9% purity (Praxair, Brazil), a heating tape, a Teledyne Isco syringe pump, model 500D, a HPLC liquid pump Lab Alliance Series III, the stainless steel extractor and two thermostatic baths. The first brath brand Julabo, model F32, used to cool the solvent prior to entering the pump and the second bath brand Quimis, scientific devices Ltd., Model Q214-M2, 220V, 2100W, to maintain the extractor heated in the desired temperatures.

Antioxidant activity was determined by the method of determination of phenolic compounds, Folin-Ciocalteu [13, 14]., And by, Method Sequestration of free radicals, DPPH - 2,2 diphenyl-1-picrylhydrazyl [15].

3. Results and discussion

The moisture content of dehydrated sweet tamarind seed was 5.00 ± 0.085%. The overall yield was determined as the fraction of compounds present in solid matrix that can be extracted by solvent in pre-established extraction conditions [16].

3.1 Extraction yields with pure CO_2

According to the presented results the highest yield obtained for the ESC of the sweet tamarind seed was 2,6218% to the condition of 250 bar and 68 F, statistically equal to the yields obtained in the same pressure and temperature of 122 F , 2,5799%, and also at the midpoint, 175 bar and 95 F, to the 5% level of significance according to Table 3.

Table 3. Global efficiency of extraction with pure CO_2

Experiments	T (F)	P (bar)	ρCO_2 $(g/cm^3)^{(1)}$	Solvent	Xo (%)$^{(2)}$
1	68	100	0,85653	CO_2	1,6223^{ab}
2	122	100	0,38535	CO_2	0,0000^{b}
3	**68**	**250**	**0,96350**	**CO_2**	**2,6218^{a}**
4	122	250	0,83497	CO_2	2,5799^{ab}
5	95	175	0,84394	CO_2	2,3591^{ab}
6	95	175	0,84394	CO_2	2,4891^{ab}
7	95	175	0,84394	CO_2	2,5671^{ab}

(1) ANGUS et al. [17], 1976;
(2) Same letters do not differ significantly ($p<0,05$).
T= temperatures; P= pressure; ρ=density; Xo= yield.

There was no extract for supercritical extraction in the conditions of 122 F and 100 bar with CO_2, due to the low density of the solvent, 0,38535 g/cm^3, which decreases the solvating power of it, however, subcritical temperature, 68 F, and supercritical pressure, 100 bar with CO_2, were extracted 1,6223% of a yellow oil, confirming the influence of the specific mass that increased to 0,85653 g/cm^3.

The vapor pressure of the solute increases with increased temperature, however, in this experiment, the solvent density had a dominant effect on the extraction process. A similar effect is observed at a pressure of 250 bar, with increased density of 0,83497 g/cm^3 to 0,9635 g/cm^3, there is an increase of approximately 2,58% to 2,62%, although not considered significant at the level 5% by the Tukey test.

These results agree with Benelli [18] (2010), who found similar results using supercritical CO_2 extraction of antioxidants and bioactive compounds of orange peel on the condition of 100 bar, the yield decreased from 1,16 ± 0.06% (w/w) to 0,84 ± 0.06% (w/w) with the elevation of temperature of 40 °C (104 F) to 50 °C (122 F) due to reduction in the density of the supercritical CO_2.

The results show an increase in the extraction yield with increasing pressure at constant temperature. At 68 F, the yield increases from 1,62% to 2,62%, around 60% more, when pressure is increased from 100 bar to 250 bar, according to Table 3. This can be explained by the increased density of the solvent with pressure, in other words, there is an increase in the solvating power of CO_2 (solubility) with the density [19].

3.2 Extraction yields with pure CO2 + ethyl alcohol

Ethanol is considered a very polar solvent due to its polarity index (5,2). This compound has been used as a cosolvent in several studies on supercritical extraction of antioxidants in small concentrations. According Benelli [18] (2010), phenolic compounds possess higher affinity for solvents of intermediate and high polarity (3,1 to 5,2). Therefore, in this work, it was decided to perform extractions combining carbon dioxide with increasing subcritical ethanol concentrations from 10% to 75% by mole fraction to evaluate yield and amount of polar compounds extracted from the seed of sweet tamarind. The obtained yields result with CO_2 combined with ethanol are in Table 4.

Table 4. Results of extraction with CO_2 + ethanol in the condition of 250 bar and 122 F

Experiments	T (F)	P (bar)	p CO_2 (g/cm^3)$^{(1)}$	Solvent	Xo (%)$^{(2)}$
8	122	250	0,83497	CO_2 + 10% ethanol	3,4759^c
9	122	250	0,83497	CO_2 + 25% ethanol	3,4373^c
10	122	250	0,83497	CO_2 + 50% ethanol	7,6214^b
11	122	250	0,83497	CO_2 + 75% ethanol	20,2695^a

(1)ANGUS et al. [17], 1976;
(2)Same letters do not differ significantly ($p<0,05$).
T= temperatures; P= pressure; ρ=density; Xo= yield.

The higher extraction yields with ethanol were obtained when it was used ethanol combined with CO_2, in a proportion of 3:1 respectively. With the use of ethanol were extracted a yellow oil mixed with a liquid colored reddish brown Figure 1.

Figure 1. Obtained **e**xtract at 250 bar/122 F with CO_2+ 75% ethanol.

It is noticed that there was no significant difference in extraction yield of the mixture CO_2 + ethanol at concentrations of 10% and 25%, this may be occurred because the mole fraction of ethanol increased, and hampered the extraction of nonpolar compounds, whereas ethanol is polar and extracts preferably polar compounds [20].

3.3 Antioxidant activity

The amount of phenolic compound of the sweet tamarind seed evaluated by the Folin Ciocaltou being represented by the number of equivalents grams of gallic acid by the amount of grams of sample. The results were obtained from the extracts obtained by extraction techniques used in accordance with Table 5.

The amount of phenolic compounds obtained with the pure supercritical extraction with CO_2 presented tendency of linear variations with the increase or decrease of temperature and pressure. The larger amount of antioxidant compounds found in this condition, was 250 bar and 122 F 25,19 ± 0,6 mg GAE/gextract, considered statistically equal to the amount found for the condition of 250 bar and 68 F, according to Tukey's test at 5% significance level, both the pressure and the temperature had a significant influence on the extraction of these compounds. This confirms the choice of the above condition for the extractions with ethanol.

Table 5. Total phenolic content (TPT), expressed as gallic acid equivalents for the extracts of the seed of sweet tamarind

Extracts	TPT[(1)] (mg EAG/g extrato)
ESC - 100 bar e 68 F	6,13[hi]
ESC – 100 bar e 122 F	There was no extract
ESC - 250 bar e 68 F	21,92[g]
ESC - 250 bar e 122 F	25,19[g]
ESC - 175 bar e 95 F	9,26[h]
ESC - 175 bar e 95 F	6,0[hi]
ESC - 175 bar e 95 F	4,76[j]
250 bar/122 F e 10% ethanol	25,6[g]
250 bar/122 F e 25% ethanol	307,36[e]
250 bar/122 F e 50% ethanol	361,42[d]
250 bar/122 F e 75% ethanol	420,8[b]

[(1)] Same letters do not differ significantly ($p<0,05$).

The extraction carried out at 250 bar and 122 F with different concentrations of CO_2 and ethanol, also presented quantities of phenolic compounds very different, as the amount of ethanol increased the amount of phenolic compounds also increased significantly.

Luzia and Jorge [11] (2010), found 49,30 mg GAE/gextrato of phenolic componds in ethanolic solution of Tamarindus indica L. seed, while Simas et al. [12] (2011), found 183,28 mg GAE/ mg of extract in the said fruit peels. These findings are among the values found in this study.

The antioxidant activity (AA) of the extracts of sweet tamarind seed was also assessed by reagent DPPH. Table 6 presents the values of antioxidant activity, on the highest concentration tested (500 mg mL) in AA%.

Table 6. Antioxidant activity by the DPPH method

Extracts	% AA (500µg/mL)[1]
ESC - 100 bar e 68 F	31,45[f]
ESC – 100 bar e 122 F	There was no extract
ESC - 250 bar e 68 F	28,21[g]
ESC - 250 bar e 122 F	31,15[f]
ESC - 175 bar e 95 F	53,91[c]
ESC - 175 bar e 95 F	45,24[d]
ESC - 175 bar e 95 F	41,90[e]
250 bar/122 F e 10% ethanol	25,13[h]
250 bar/122 F e 25% ethanol	97,18[a]
250 bar/122 F e 50% ethanol	97,77[a]
250 bar/122 F e 75% ethanol	97,61[a]

[(1)]Letras iguais não diferem significativamente ($p<0,05$).
[(1)] Same letters do not differ significantly ($p<0,05$).
AA= antioxidant activity

Increasing pressure and temperature favor the AA with pure CO_2 from 28,2 to 53,9% (500µg/mL), contributing to the solubility of polar compounds.

According to the results, the samples which had the highest antioxidant activities were extracted with ethanol at a concentration of 25% and 75%. This can be attributed to the polarity of the ethanol that solubilize polar compounds such as, among them, phenolic compounds. This result contributes to the extract of sweet tamarind seed making it a potential antioxidant to be tested in foods.

4. Conclusion

The mass yield obtained for the ESC of sweet tamarind seed with pure CO_2 was influenced by temperature as much as pressure. It was observed that the density of the solvent had a dominant effect in this experiment, and that the higher the density the greater the yield of the extract. Increased pressure also positively influenced the yield of the extract.

The use of ethanol in the extraction process significantly influenced the mass yield and in the antioxidant quality of extracts.

The TPT effectively increased with the increasing of ethanol concentration and antioxidant activity showed no difference in concentrations of 25%, 50% and 75%.

Therefore, the ethanol extracts of the seed of the sweet tamarind, obtained by supercritical technology with solvent mixture, are potential sources of natural antioxidants for the food industry.

Acknowledgements

We thank the Research Foundation of the State of Pernambuco for the financial support and the Tiradentes University for technical support to this research.

References

[1] P. C. Pereira; B. Melo; A. A. Franzão; P. R. B. Alves, The planting of tamarind (Tamarindus indica L.). I Week Course of Environmental Engineering. Institute of Agricultural Sciences, Federal University of Uberlândia – Campus Umarama – MG, 2011.

[2] S. Luengthanaphol; D. Mongkholkhajornsilp; S. Douglas; P. L. Douglas; L. Pengsopa; S. Pongamphai, Extraction of antioxidants from sweet Thai tamarind seed coat- preliminary experiments. Journal of Food Engineering, Essex, v. 63, n. 3, p. 247-253, 2004.

[3] R. V. dos S. Carvalho; J. S. da Silva; H. N. S. Souza; M. G. C. Guimarãos; V. R. M. dos Santos; L. C. Azevêdo, Characterization of a New Variety of Tamarind (Tamarindus indica) Grown In Bahia. IV Congress of Research and Innovation Network North and Northeast Technical Education. Belém-PA, 2009.

[4] Brasil, Ministry of Health. Ordinance nº. 540, the 27 the ouctober the 1997. Official Gazette the Union, 28 of ouctober 1997.

[5] T. A. Pereira; D. Rettori, Comparison of the antioxidant capacity of different brands of green teas. In: Journey of Initiation Scientific and Technological Uniban, 2008, São Paulo. Annals...Sao Paulo: UNIBAN, 2008. p. 1-3.

[6] E. Melo de A.;M. I. S. Maciel; V. L. A. G. Lima, Fruit antioxidant capacity. Brazilian Journal of Pharmaceutical Sciences, vol. 44, n. 2, 2008.

[7] V. C. Ramalho; N. Jorge, Photo Antioxidants. New chemistry, Vol. 29 n°. 4 São Paulo. July/Aug. 2006

[8] M. T. L. P. Peres; J. R. R. Lopes; C. B. da Silva; A. C. S. Cândido; S. C. Hess; E. Simionatto; C. A. L. Cardoso; P. H. Simas, Content of phenols and Antioxidant Activity of Fruits of the Cerrado. Brazilian Chemical Society (SBQ). 34ª. Annual Meeting of the Brazilian Chemical Society. Florianópolis, 2011.

[9] T. Tsuda; M. Watanable; K. Ohshima; A. Yamamoto; S. Kawakishi; T. Osawa, Antioxidative components isolated from the seed of tamarind (Tamarindus indica L.). J. Agric. Food Chem., Washington, v.42, p. 2671-2674, 1994.

[10] J. Siddhuraju, *LWT* 2007, *40*, 982

[11] D. M. M. Luzia; N. Jorge, Antioxidant activity, fatty acid profile and tocopherols of Tamarindus indica L. seeds. Ciênc. Tecnol. Aliment., Campinas, 31(2): 497-501, abr.-jun. 2011

[12] P. H. Simas; E. Simionatto; M. R. Cabral; J. T. Facco; R. P. Andreio; C. B. da Silva; M. T. L. P. Peres, Research on phenolic content and antioxidant activity of fruits of MS. Disp. em: http://periodicos.uems.br/novo/index.php/enic/article/view/652/582. Acess: 03 of nov. 2012.

[13] V.L. Singleton; J.A. Rossi, Colorimetric of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal Enol. Vitic. v. 16, p. 144-58, 1965.

[14] W. Peschel; F. Sánchez-Rabaneda; W. Diekmann; A. Plescher; I. Gartzía; D. Jiménez; R. Lamuela-Raventós; S. Buxaderas; C. Codina, An industrial approach in the search of natural antioxidants from vegetable and fruit wastes.

Food Chemistry, v. 97, p. 137-50, 2006.

[15] L. L. Mensor; F. S. Menezes; G. G. Leitão; A. S. Reis; T. C. Santos; C. S. Coube; S. Leitão, Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res., v. 15, p. 127-30, 2001.

[16] D. C. M. N. Silva, Experimental determination of process parameters on supercritical extraction of gorce essential oil (Baccharis trimera Less). Florianópolis: UFSC, 2004. Dissertação (Mestrado em Engenharia de Alimentos), Departamento de Engenharia Química e Engenharia de Alimentos, Universidade Federal de Santa Catarina, 2004.

[17] S. Angus; B. Armstrong; K. M. De Reuck International thermodynamic tables of the fluid state: Carbon dioxide. Oxford: Pergamon Press, 1976.

[18] P. Benelli, Adding Value to marc Orange (Citrus sinensis L. Osbeck) Upon Obtaining Bioactive Extracts using Different Extraction Techniques. Florianópolis: UFSC, 2010. Dissertation (Masters in Food Engineering), Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina, 2010.

[19] G. Brunner, Gas Extraction: An introduction to fundamentals of supercritical fluids and the applications to separation process. Darmastadt, Alemanha: Steikopff, Springer, 1994.

[20] D. A. de. Oliveira, Phytochemical characterization and biological extracts from grape pomace (Vitis vinifera) varieties Merlot and Syra. Florianópolis: UFSC, 2010. Dissertation (Masters in Food Engineering), Department of Chemical Engineering and Food Engineering, Federal University of Santa Catarina, 2010.