Spilanthol Content in the Extract Obtained by Supercritical CO 2 at Different Storage Times of Acmella Oleracea L.: SPILANTHOL AT DIFFERENT STORAGE TIMES OF JAMBU

Article *in* Journal of Food Process Engineering · July 2016

DOI: 10.1111/jfpe.12441

CITATIONS
10

READS
1,393

7 authors, including:

Alan Franco Barbosa
Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso Campus Sorriso
17 PUBLICATIONS **228** CITATIONS
SEE PROFILE

Cristiane Pereira
Universidade de Vassouras
85 PUBLICATIONS **134** CITATIONS
SEE PROFILE

Marisa Mendes
Federal Rural University of Rio de Janeiro
120 PUBLICATIONS **927** CITATIONS
SEE PROFILE

Raul Carvalho Junior
Federal University of Pará
28 PUBLICATIONS **621** CITATIONS
SEE PROFILE

Journal of
Food Process Engineering

Journal of Food Process Engineering ISSN 1745–4530

SPILANTHOL CONTENT IN THE EXTRACT OBTAINED BY SUPERCRITICAL CO_2 AT DIFFERENT STORAGE TIMES OF ACMELLA OLERACEA L.

ALAN FRANCO BARBOSA[1,8], CRISTIANE DE SOUZA SIQUEIRA PEREIRA[2], MARISA FERNANDES MENDES[3], RAUL NUNES DE CARVALHO JUNIOR[4], MÁRIO GERALDO DE CARVALHO[5], JOSÉ GUILHERME SOARES MAIA[6] and ARMANDO UBIRAJARA OLIVEIRA SABAA-SRUR[1,7]

[1]Departamento De Tecnologia De Alimentos, Universidade Federal Rural Do Rio De Janeiro, BR 465, Km 7, Seropédica, Rio De Janeiro 23897-000, Brazil
[2]Escola De Química, Universidade Federal Do Rio De Janeiro, Av. Athos Da Silveira Ramos 149, Ilha Do Fundão, Rio De Janeiro 21941-902, Brazil
[3]Departamento De Engenharia Química, Universidade Federal Rural Do Rio De Janeiro, BR 465, Km 7, Seropédica, Rio De Janeiro 23897-000, Brazil
[4]Faculdade De Engenharia De Alimentos, Universidade Federal Do Pará, Rua Augusto Corrêa S/N, Guamá, Belém, Pará, 66075-900, Brazil
[5]Departamento De Química, Universidade Federal Rural Do Rio De Janeiro, BR 465, Km 7, Seropédica, Rio De Janeiro 23897-000, Brazil
[6]Programa De Pos-Graduação Em Recursos Naturais Da Amazônia, Universidade Federal Do Oeste Do Pará, Rua Vera Paz, S/N, Salé, Santarém, Pará 68035-110, Brazil
[7]Departmento De Nutrição Básica E Experimental, Universidade Federal Do Rio De Janeiro, Av. Carlos Chagas Filho 373, Ilha Do Fundão, Rio De Janeiro 21941-902, Brazil

[8]Corresponding author.
TEL: +55-21-979919350;
FAX: +55-21-26821023;
EMAIL: alanfbarbosa@yahoo.com.br

Received for Publication December 22, 2015
Accepted for Publication May 31, 2016

doi:10.1111/jfpe.12441

ABSTRACT

The effect of the storage time on spilanthol content in the extract from Jambu obtained by supercritical CO_2 was evaluated on respect of their chemical properties. Lyophilized Jambu and partially dehydrated with cold air circulation was stored in an oven at 40°C for 195 days. After different storage times (0, 21, 41, 89 and 195 days), the samples were submitted to supercritical fluid extraction at the same operational condition of 300 bar and 40°C. The obtained extracts were analyzed by ^{1}H and ^{13}C NMR and GC-MS. The extract from lyophilized Jambu and from different storage times provided the respective spilanthol content, % (storage days): 1.07 (lyophilized); 0.68 (0); 0.41 (21); 0.31 (41); 0.41 (89) and 0.42 (195). These determinations allowed the identification of the constant presence of spilanthol as the major metabolite in the extracts, regardless of storage time, revealing the viability of the market for this product in chemical composition.

PRACTICAL APPLICATIONS

This paper showed the presence of spilanthol in all the extracts from a supercritical fluid, despite of the different storage times that the raw material (Jambu) was submitted, proving that it did not suffer degradation. This result is significant because spilanthol is considered the most special metabolite present in Jambu. Moreover, it has many biological properties and confers flavor with tingling and salivation during consumption.

INTRODUCTION

Popularly known as Jambu, *Acmella oleracea* L. is a native plant from Amazon, often used as a flavor in popular dishes of northern Brazil, having as well application in popular medicine for the treatment of stomatitis, colds and as an analgesic (Nascimento *et al.* 2013). The plant has important chemical properties that improve the interest of the pharmaceutical industry, especially due to the presence of a high-value component, known as spilanthol (Borges *et al.* 2012).

The spilanthol is an outstanding representative of the *N*-alkylamides, which high biomedical interest has increased in the last two decades (Boonen *et al.* 2012; Monroe *et al.*

2016). This group of bioactive molecules acts in the protection of plants and biocide products, functional foods, cosmetics and pharmaceuticals components (Veryser *et al.* 2014).

The spilanthol is present in several species of the *Acmella* genus in Asteraceae, such as *Acmella oleracea* L. and *Acmella affinis* Hook & Arn. Spilanthol can also be found in *Acmella ciliata* (Kunth) Cass., *Acmella oppositifolia* (Lam.) R.K. Jansen, *Acmella radicans* (Lam.) R.K. Jansen, *Acmella brachyglossa* Cass., *Acmella paniculata* (Wall. ex DC.) R.K. Jansen, and *Acmella uliginosa* (Sw.) Cass. (Chung *et al.* 2008; Prachayasittukal *et al.* 2013).

This amide was obtained from *A. oleracea* (Jacobson 1957) and synthesized by Ikeda and colleagues (1984). There are differences between species, methods and solvents used in the extraction, depending on the origin of species, soil, climate and growth phase that may influence the concentration of this amide in *Acmella* species.

Commercially, 5 mg of spilanthol costs about US$200.00 (2014, Chromadex company, Germany). The spilanthol is described as viscous oil, burning and light yellow color (Jacobson 1957).

Costa *et al.* (2013) improved the spilanthol extraction using a microwave with ethanol and hexane (3:7) as the solvent. The oil was extracted at 50°C, for 30 min and analyzed by gas chromatography to confirm the presence of spilanthol. The results showed the presence of spilanthol with a high determination coefficient of 99.8%.

Dias *et al.* (2012) studied the extracts from leaves, stems and flowers of Jambu, obtained by supercritical CO_2 followed by another extraction with ethanol, water and mixtures as cosolvents. The results showed that the flowers are richer in spilanthol, justifying the higher antioxidant/phenolic as well as the largest antiinflammatory activity. The yield of spilanthol in flowers extracts in this study was 65.4%, followed by 47.3% in the stems and 19.7% in the leaves. They also reported that the extraction with supercritical CO_2 has proved a particulars electivity for spilanthol, especially in the case of flowers. It is one of the advantages of the process in question, which commonly uses carbon dioxide as a solvent because it is nontoxic, low cost and has high volatility, providing an extract free of organic solvent.

Among the spilanthol activities, it can be mentioned the acaricide activity (Castro *et al.* 2014), used for skin diseases such as eczema (Boonen *et al.* 2010b), antiwrinkle effect (Demarne and Passaro 2005), analgesic (Rios *et al.* 2007), antimalarial (Spelman *et al.* 2011), fungistatic and bacteriostatic (Molina-Torres *et al.* 2004), activity against *Aedes aegypti* and corn earworm (Ramsewak *et al.* 1999), activity diuretic (Ratnasooriya *et al.* 2004), antiseptic, immune stimulation (Rojas *et al.* 2006), inactivation of tyrosinase enzyme (Barbosa *et al.* 2016b), induces secretion of saliva (Sharma *et al.* 2011) and resides insecticidal activity (Kadir *et al.* 1989; Moreno *et al.* 2012; Sharma *et al.* 2012). More details about the occurrence, extraction, chemistry and biological activities of spilanthol were previously reported (Barbosa *et al.* 2016a).

Jambu is an edible vegetable, with spilanthol as its main compound. Because of this, it is fundamental to assess the content of the amide in function of time, product storage conditions, temperature storage and packaging due to their interference in the presence of substances in food. The presence of spilanthol even in extreme storage conditions would indicate the viability of Jambu commercialization.

Because of the evidences cited above, as the activities of the high added-value component and its contributions to the food, cosmetic and pharmaceutical areas, this work has as goal the evaluation of the effect of Jambu's storage time in the content of spilanthol extracted using supercritical CO_2 as a solvent, seeking to prove the maintenance of spilanthol.

MATERIALS AND METHODS

Jambu Samples

The Jambu (*A. oleracea* L.) was collected in Igarapé-Açu (Pará State), in the following coordinates: 01°07′33″S and 47°37′27″W (Oliveira *et al.* 2011). A voucher specimen (MG205534) was deposited at the Emilio Goeldi Paraense Museum, Belem, Brazil. The plant flowers, leaves and stems were dried and comminuted. It was used a mini-processor Black & Decker, model HC31T, Brazil, to grind the Jambu. The particle size distribution was analyzed using a series of sieves (8–65 mesh) under mechanical stirring (Bertel, model VP-01, Brazil), and the average particle diameter was calculated based on the Sauter mean diameter (Massarani 1984). The Sauter mean diameter ($d = (1/\Sigma i x_i/d\#)$) is calculated based on the summation of the ratio considering the mass sample fraction (x_i) presented in each sieve that represents different diameters (d).

Drying Process

For Jambu drying, initially, the vegetable was washed with water to remove soil residues. Then, the roots were removed with stainless knives, eliminating parts of the vegetable which were torn, crumpled and with darkened edges. The raw materials were sanitized by immersion for 10 min in a solution of 200 ppm (mg L^{-1}) of free residual chlorine (FRC), derived from sodium hypochlorite with 10% purity. Then, the last rinse was performed with the soaking of a solution of 5 ppm (mg L^{-1}) FRC for 10 min with subsequent drainage of water.

The cold-drying process was carried out in the climatized room with air conditioning (Midea, model MS2E-18CR, Brazil), at 25°C and using a dehumidifier (Arsec, model 160,

TABLE 1. YIELDS OF JAMBU EXTRACTS (*ACMELLA OLERACEA* L.) AND SPILANTHOL AND HUMIDITY VALUES FOR DRY JAMBU EXTRACTION WITH SUPERCRITICAL CO_2

	Humidities (%)	Global yields (% d.b.)	Relative % area of spilanthol	Spilanthol yields (%, d.b.)
Lyophilized Jambu	–	1.41	76.21	1.07
Dry Jambu – (0 day)	10.07 ± 0.03^{a}	1.61	42.14	0.68
Dry Jambu – (21 days)	6.11 ± 0.27^{b}	0.41	100	0.41
Dry Jambu – (41 days)	16.88 ± 0.36^{c}	0.31	100	0.31
Dry Jambu – (89 days)	5.44 ± 0.11^{b}	0.41	100	0.41
Dry Jambu – (195 days)	2.07 ± 0.68^{d}	0.88	47.98	0.42

Different letters between rows indicate significant statistically difference ($P < 0.05$).

Brazil), in a 4.0 m^2 room that remained closed during the drying procedure. The cold air dried Jambu presented moisture level of 10.07% (Table 1).

The fresh Jambu was previously lyophilized before extraction with supercritical CO_2. The lyophilization was done in Alpha 1-4/LDC-1 M, Christ, Osterode, Germany. The fresh Jambu was lyophilized for 72 h at −51°C and 0.011 mbar.

Storage of Jambu

The monitoring of spilanthol content was performed with 25 g of dried Jambu samples (flowers, leaves and stems) packed in low-density polyethylene bags and aluminum foil to prevent photo-oxidation of the sample. The Jambu sample was stored in an oven at 40°C during 0, 41, 61, 89 and 195 days. The temperature used was chosen based on a value above the common temperature of food storage, accelerating the commercial shelf life of the product. The storage time was selected based on the availability of supercritical CO_2 extractor. For each time, it was determined the humidity according to AOAC 934.06 method (AOAC 1997). All experiments were performed in triplicate, and the results obtained were expressed as means of standard deviations (±SD). It was used the Tukey test at 5% level of significance ($P > 0.05$) to compare the media, using the software BioStat 5.0 (Ayres *et al.* 2007). The supercritical fluid technology has been used to obtain Jambu extract at each time of storage, and the spilanthol content was also monitored through all the storage time (NMR ^{1}H and ^{13}C and GC/MS). The samples were stored at 40°C, slightly above the usual storage temperature for food storage, to simulate extreme conditions for the product storage.

Experimental Procedure for SFE

The experiments were performed in Applied Thermodynamics and Biofuel Laboratory (UFRRJ, Seropédica, Brazil) according to the methodology described by Dias *et al.* (2012). The extractor of this equipment has a capacity of 4.2×10^{-5} m^3. The solvent used was carbon dioxide 99.9% of minimum purity, White Martins LTDA, Rio de Janeiro, Brazil. The yield of the extracts was obtained with lyophilized and cold air dried Jambu. Initially, the extractor was filled with, approximately 0.005 kg of solid materials of Jambu. The experimental conditions used were: temperature of 40°C, the pressure of 300 bars and the solvent flow rate used was 2.73×10^{-4} kg/s. The overall extraction curve (OEC) was obtained using the operational conditions cited above. Samples were collected at 19 intervals of 10 min.

Composition Analysis

The material was analyzed by gas chromatograph coupled to a mass spectrometer GC/MS Shimadzu, model QP-2010 Plus, Japan, and by ^{1}H and ^{13}C NMR spectra data (Bruker, model advance III, EUA). Dichloromethane 99.9%, HPLC grade, Sigma-Aldrich Inc., EUA, was used as the solvent in GC/MS analysis and $CDCl_3$ 99%, Sigma-Aldrich Inc., USA, was used as the solvent in NMR analysis. The GC/MS was equipped with a Factor Four/VF-5ms fused-silica capillary column (30 m × 0.25 mm × 0.25 μm film thickness), using helium as carrier gas at 1 mL/min. The initial oven temperature was 100°C, and after being constant for 40 min, it was increased at a rate of $10°C \cdot min^{-1}$ to 290°C, with a final isotherm (300°C) in 20 min. The sample injection volume was 1 μL (1:50 split mode). The injector and detector temperatures were both 300°C. The mass spectra were obtained in a range of *m/z* 10–300, by the electron impact technique at 70 eV. The quantitative analysis of the samples' chemical composition was carried out in a HP 5890 Series II gas chromatograph with flame ionization detector (FID), using the same operational conditions and the same type of column as in the GC/MS analysis, with exception of the injector and detector temperatures that were of 250 and 300°C, respectively.

The percentage of each constituent was calculated by the integral area under the respective peaks about the total area of all the sample constituents. The identification of the major constituent was made based on the information obtained from the mentioned analytic methods, together with the data generated by comparison of the nuclear magnetic resonance spectra of hydrogen, ^{1}H NMR (Bruker 500 MHz spectrometer), and carbon, ^{13}C NMR (Bruker 125 MHz spectrometer).

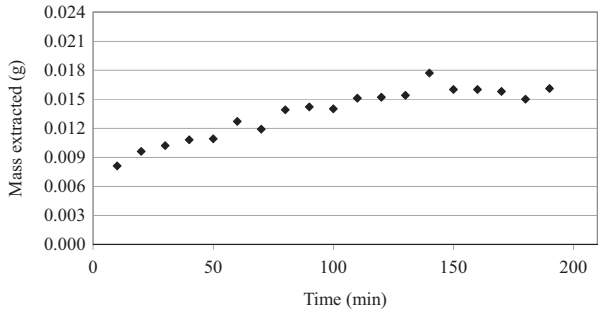

FIG. 1. EXPERIMENTAL EXTRACTION CURVE OF JAMBU OBTAINED AT 300 BAR AND 40°C, WHICH HAD THE HIGHEST SPILANTHOL CONTENT (FIFTH EXTRACTION)

RESULTS AND DISCUSSION

Jambu Samples

The average diameter of the samples ranged from 0.47 to 0.66 mm. Table 1 shows the humidity values of the dried jambu in each extraction using supercritical CO_2.

During the Jambu storage, it was observed the decrease of the moisture. There was a significant difference at 5% level of significance by Tukey test, as the moisture content in all extractions had the different content, except in extractions 2 and 4 (Table 1). All samples were stored at 40°C, and therefore, the behavior was expected from the results shown in Table 1. Moreover, the packaging is permeable to oxygen and presents a low barrier to water vapor and because of that, moisture loss favors the quality of the product.

SFE Global Yield. According to Dias *et al.* (2012), based on other published studies (Stashenko *et al.* 1996; Sun *et al.* 2002), high temperatures and low pressures are essential for obtaining a high content of spilanthol in the extracts. Some studies even indicate that increasing the solvent density does not involve an increase in the content of spilanthol. Because of this and the operational conditions already studied by other authors (340–355 bar, 45–60°C) and also cited in Dias *et al.* (2012), it was chosen the pressure of 300 bar and temperature of 40°C, in order to have a comparison with the standard literature, although the objectives are different.

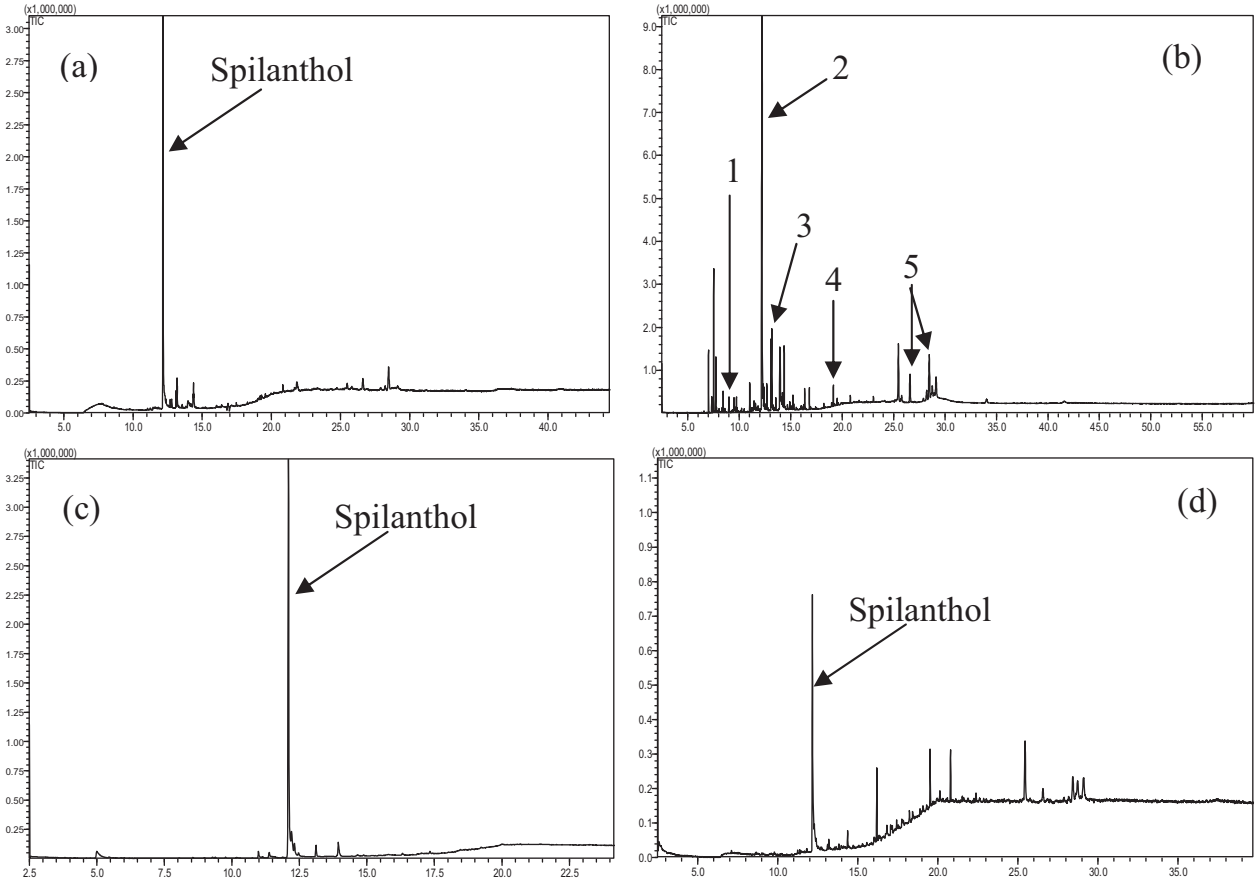

FIG. 2. GC-MS CHROMATOGRAM OF LYOPHILIZED JAMBU EXTRACT (a), FIRST (b), SECOND, THIRD, FOURTH (c) AND FIFTH (d) EXTRACTION JAMBU OBTAINED BY SUPERCRITICAL CO_2.

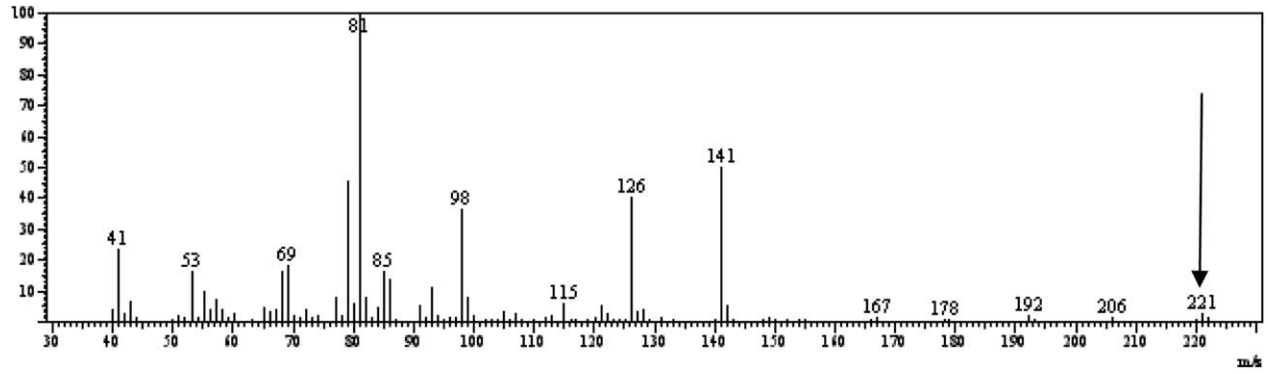

FIG. 3. MASS SPECTRUM OF THE SPILANTHOL (1).

The first extraction of dried Jambu and the extraction of lyophilized jambu had higher yields (Table 1), probably due to the higher concentration of fatty matter (Fig. 2b). As the storage time increases, the fatty matter decreases, due to oxidation of these nutrients, and probably increases the concentration in spilanthol in the extract.

The loss of these nutrients can be justified by the high storage temperature above the usual food storage temperature. Moreover, it has been used an oxygen permeable container, not preventing the oxidation, despite of the samples did not suffer photo-oxidation since the containers were covered with foil. Also, the lipids are highly unstable substances and, because of the high temperature, they are subjected to drastic changes in their sensory characteristics, functional and nutritional value (Guerra and Lajolo 2005). The total yields after different storage times are shown in Table 1.

Figure 1 shows the overall extraction curve (cumulative mass versus extraction time) obtained for the dried Jambu extract (fifth), which had the highest spilanthol content during storage of the product.

Composition Analysis

Table 1 shows the relative area (%) and the spilanthol yields of the evaluated samples. The spilanthol is the major metabolite in all extractions, demonstrating the importance of this active ingredient. It is important to note the presence of another amide, *N*-(2-methylbutyl)-(2*E*,6*Z*,8*E*)-decatrienamide, which has the chemical structure similar to spilanthol. This compound, also known as homospilanthol, is the second most abundant *N*-alkylamide in the *Spilanthes* ethanolic extract (9.04% of the total amount of *N*-alkylamides) (Boonen *et al.* 2010a,b).

Comparing spilanthol content in lyophilized and dried Jambu after 195 days of storage (Table 1), it can be observed that the spilanthol is still present against lyophilized sample by 39%.

The chromatograms of the analysis by gas chromatography-mass spectrometry (GC/MS) showed majority peaks with retention times (RT, in minutes) at 12.169, 12.225 and 12.158 for lyophilized jambu extract, first and fifth extraction, respectively (Fig. 2).

In the chromatogram number 2, Fig. 2b, it can be observed the main peak of extract from dried Jambu (first extraction). Besides spilanthol, the sample has other bioactive compounds such as squalene, normally present in fish oils and other secondary metabolites present in plants such as lupelila acetate (0.05%) and caryophyllene oxide (0.01%).

After 195 days of storage, the spilanthol was still present in the dried Jambu samples in 63%. The spilanthol remained in the product because of its chemical stability due to its chemical structure; high molecular weight (221 g/mol) and high boiling point (165°C) (Jacobson 1957).

The second, third and fourth extraction by supercritical CO_2 of dried Jambu showed 100% of spilanthol in GC/MS analysis, presenting 87%, 88% and 87% of similarity to the library, respectively.

The main component present in the extract was identified by the peak at 12.09 min (second, third and fourth extraction) in the GC chromatogram (Fig. 2c) which presented the mass spectrum (Fig. 3) with peaks values at [*m/z* (ion, %)]: 221 (M^+, 2), 141 (M-C_6H_8, 50), 126 ($[C_7H_{12}NO]^+$, 40), 98 ($[C_5H_8NO]^+$, 35), 81 ($C_6H_9^+$, 100)], and they are in accordance with the spilanthol structure. Besides these data, the spilanthol structure was confirmed by ^1H NMR and ^{13}C NMR analysis, and with the literature data (Nakatani and Nagashima 1992).

CONCLUSIONS

The presence of spilanthol as the major metabolite in the extracts, independently of the storage time, revealed the chemical feasibility of the commercialization of dried Jambu, maintaining the main substance even 195 days of storage under extreme conditions of temperature and packaging.

ACKNOWLEDGMENTS

The authors are grateful to FAPERJ, CNPQ and to CAPES for scholarships and financial support.

REFERENCES

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS (AOAC), 1997. Association of official analytical chemists. In *Official Methods of Analysis of AOAC International*, 16th Ed., 3rd revision (P. Cuniff ed.), AOAC International, Gaithersburg, Maryland.

AYRES, M., AYRES,M., JR, AYRES, D.L. and SANTOS, A.S.D. 2007. *BioEstat 5.0 – Aplicações estatísticas nas áreas das cïencias biológicas e médicas*, Sociedade Civil Mamirauá/MCT-CNPq, Manaus, Brazil.

BARBOSA, A.F., CARVALHO, M.G.D., SMITH, R.E. and SABAA-SRUR, A.U.O. 2016a. Spilanthol: Occurrence, extraction, chemistry and biological activities. Braz. J. Pharmacognosy *26*, 321–325.

BARBOSA, A.F., SILVA, K.C.B., OLIVEIRA, M.C.C.D., CARVALHO, M.G.D. and SABAA-SRUR, A.U.O. 2016b. Effects of *Acmella oleracea* methanolic extract and fractions on the tyrosinase enzyme. Braz. J. Pharmacognosy, in press.

BOONEN, J., BAERT, B., BURVENICH, C., BLONDEEL, P., DE SAEGER, S. and DE SIELGELEER, B. 2010a. LC-MS profiling of *N*-alkylamides in *Spilanthes acmella* extract and the transmucosal behaviour of its main bio-active spilanthol. J. Pharm. Biomed. Anal. *53*, 243–249.

BOONEN, J., BAERT, B., ROCHE, N., BURVENICH, C. and DE SPIEGELEER, B. 2010b. Transdermal behaviour of the *N*-alkylamide spilanthol (affinin) from *Spilanthes acmella* (compositae) extracts. J. Ethnopharmacol. *127*, 77–84.

BOONEN, J., BRONSELAER, A., NIELANDT, J., VERYSER, L., DE TER, G. and DE SPIEGELEER, B. 2012. Alkamid database: Chemistry, occurrence and functionality of plant *N*-alkylamides. J. Ethnopharmacol. *142*, 563–590.

BORGES, L.D.S., VIEIRA, M.A.R., MARQUES, M.O.M., VIANELLO, F. and LIMA, G.P.P. 2012. Influence of organic and mineral soil fertilization on essential oil of *Spilanthes oleracea* cv. Jambuarana. Am. J. Plant Physiol. *7*, 135–142.

CASTRO, K.N.C., LIMA, D.F., VASCONCELOS, L.C., LEITE, J.R.S.A., SANTOS, R.C., PAZ NETO, A.A. and COSTA-JÚNIOR, L.M. 2014. Acaricide activity *in vitro* of *Acmella oleracea* against *Rhipicephalus microplus*. Parasitol. Res. *113*, 3697–3701.

CHUNG, K.F., KONO, Y., WANG, C.M. and PENG, C.I. 2008. Notes on *Acmella* (asteraceae: heliantheae) in Taiwan. Bot. Stud. *49*, 73–82.

COSTA, S.S., ARUMUGAM, D., GARIEPY, Y., ROCHA, S.C.S. and RAGHAVAN, V. 2013. Spilanthol extraction using microwave: Calibration curve for gas chromatography. Chem. Eng. Trans. *32*, 1783–1788.

DEMARNE, F. and PASSARO, G. 2005. Use of an *A*cmella oleracea extract for the *botulinum* toxin-like effect thereof in an anti-wrinkle cosmetic composition. US Patent No. 7,531,193 B2.

DIAS, A.M.A., SANTOS, P., SEABRA, I.J., CARVALHO, R.N., JR, BRAGA, M.E.M. and SOUSA, H.C.D. 2012. Spilanthol from *Spilanthes acmella* flowers, leaves and stems obtained by selective supercritical carbon dioxide extraction. J. Supercrit. Fluids *61*, 62–70.

GUERRA, N.B. and LAJOLO, F.M. 2005. Ação antioxidante de especiarias face diferentes atividades de água. Food Sci. Technol. *25*, 45–50.

IKEDA, Y., UKAI, J., IKEDA, N. and YAMAMOTO, H. 1984. Facile routes to natural acyclic polyenes syntheses the spilanthol and trail pheromone for termite. Tetrahedron Lett. *25*, 5177–5180.

JACOBSON, M. 1957. The structure of spilanthol. Chem. Indus. *2*, 50–51.

KADIR, H.A., ZAKARIA, M.B., KECHIL, A.A. and AZIRUN, M.S. 1989. Toxicity and electrophysiological effects of *Spilanthes acmella* Murr. Pest. Sci. *25*, 329–335.

MASSARANI, G. 1984. Problemas em Sistemas Particulados. In (E. Blucher, ed.).

MOLINA-TORRES, J., SALAZAR-CABRESA, C.J., ARMENTA-SALINAS, C. and RAMÍREZ-SÁNCHEZ, E. 2004. Fungistatic and bacteriostatic activities of alkamides from *Heliopsis longipes*roots: Affinin and reduces amides. J. Agric. Food Chem. *52*, 4700–4704.

MONROE, D., LUO, R., TRAN, K., RICHARDS, K.M., BARBOSA, A.F., CARVALHO, M.G.D., SABAA-SRUR, A.U.O. and SMITH, R.E. 2016. LC-HRMS and NMR analysis of lyophilized *Acmella oleracea* capitula, leaves and stems. Nat. Prod. J. *6*, 10–11.

MORENO, S.C., CARVALHO, G.A., PICANÇO, M.C., MORAIS, E.G.F. and PEREIRA, R.M. 2012. Bioactivity of compounds from *Acmella oleracea* against *Tuta absoluta* (meyrick) (lepidoptera: gelechiidae) and selectivity to two non-target species. Pest Manage. Sci. *68*, 386–393.

NAKATANI, N. and NAGASHIMA, M. 1992. Pungent alkamides from *Spilanthes acmella* L. Var. Oleraceae Clark. Biosci. Biotechnol. Biochem. *56*, 759–762.

NASCIMENTO, A.M., SOUZA, L.M.D., BAGGIO, C.H., WERNER, M.F.D.P., MARIA-FERREIRA, D., SILVA, L.M.D., SASSAKI, G.L., GORIN, P.A.J., IACOMINI, M. and CIPRIANI, T.R. 2013. Gastroprotective effect and structure of a rhamnogalacturonan from *Acmella oleracea*. Phytochemistry *85*, 137–142.

OLIVEIRA, A., AMARAL, A.J., ANDRADE, J., NASCIMENTO, V., MENDES, K. and REIS, J. 2011. Desenvolvendo construções de apriscos na agricultura familiar. Cad. Agroecol. *6*, 1–4.

PRACHAYASITTUKAL, V., PRACHAYASITTUKAL, S., RUCHIWARAT, S. and PRACHAYASITTUKAL, V. 2013. High therapeutic potential of *Spilanthes acmella*: A review. EXCLI J. *12*, 291–312.

RAMSEWAK, R.S., ERIKCSON, A.J. and NAIR, M.G. 1999. Bioactive *N*-isobutylamides from the flower buds of *Spilanthes acmella*. Phytochemistry *51*, 729–732.

RATNASOORIYA, W.D., PIERIS, K.P.P., SAMARATUNGA, U. and JAYAKODY, J.R.A.C. 2004. Diuretic activity of *Spilanthes acmella*, flowers in rats. J. Ethnopharmacol. *91*, 317–320.

RIOS, M.Y., AGUILAR-GUADARRAMA, A.B. and GUTIERREZ, M.D. 2007. Analgesic activity of affinin, an alkamide from *Heliopsis longipes* (compositae). J. Ethnopharmacol. *110*, 364–367.

ROJAS, J.J., OCHOA, V.J., OCAMPO, S.A. and MUÑOZ, J.F. 2006. Screening for antimicrobial activity of ten medicinal plants used in colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. BMC Complement. Altern. Med. *6*, 2–7.

SHARMA, A., KUMAR, V., RATTAN, R.S., KUMAR, N. and SINGH, B. 2012. Insecticidal toxicity of spilanthol from *Spilanthes acmella* murr. Against *plutella xylostella* L. Am. J. Plant Sci. *3*, 1568–1572.

SHARMA, V., BOONEN, J., CHAUHAN, N.S., THAKUR, M., DE SPIEGELEER, B. and DIXIT, V.K. 2011. *Spilanthes acmella* ethanolic flower extract: LC-MS alkylamide profiling and its effects on sexual behavior in male rats. Phytomedicine *18*, 1161–1169.

SPELMAN, K., DEPOIX, D., MCCRAY, M., MOURAY, E. and GRELLIER, P. 2011. The traditional medicine *Spilanthes acmella*, and the alkylamides spilanthol and Undeca-2*E*-ene-8,10-diynoic acid isobutylamide, demonstrate *in vitro* and *in vivo* antimalarial Activity. Phytother. Res. *25*, 1098–1101.

STASHENKO, E.E., PUERTAS, M.A. and COMBARIZA, M.Y. 1996. Volatile secondary metabolites from *Spilanthes americana* obtained by simultaneous steam distillation-solvent extraction and supercritical fluid extraction. J. Chromatogr. A *752*, 223–232.

SUN, L., REZAEI, K.A., TEMELLI, F. and OORAIKUL, B. 2002. Supercritical fluid extraction of alkylamides from *Echinacea angustifolia*. J. Agric. Food Chem. *50*, 3947–3953.

VERYSER, L., WYNENDAELE, E., TAEVERNIER, L., VERBEKE, F., JOSHIB, T., TATKEB, P. and DE SPIEGELEER, B. 2014. *N*-alkylamides: From plant to brain. Funct. Foods Health Dis. *4*, 264–275.