CHEMICAL ENGINEERING TRANSACTIONS
VOL. 44, 2015
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Riccardo Guidetti, Luigi Bodria, Stanley Best

ISBN 978-88-95608-35-8; **ISSN** 2283-9216

Volatile Coumponds in Passion Fruit Seed Oil (Passiflora setacea BRS Pérola do Cerrado and Passiflora alata BRS Doce Mel)

Regina Cássia Mattos de Paula*[a], Antônio Gomes Soares[b], Suely Pereira Freitas[a]

[a]Universidade Federal do Rio de Janeiro (UFRJ), Av. Athos da Silveira Ribeiro, 164 Bloco I, LADEQ, Ed. Centro de Tecnologia, Escola de Química, Cidade Universitária, Ilha do Fundão, Rio de Janeiro-RJ, Brazil
[b]Embrapa Agroindústria de Alimentos, Av. das Américas, 29501 Guaratiba, Rio de Janeiro-RJ, Brazil
reginacassiamattos@yahoo.com.br

The use of seed byproducts from fruit processes has contributed to increase the supply of vegetable oils rich in bioactive compounds regarding consolidated cosmetics and functional foods. The aim of this study was to identify the volatile compounds present in the species *P. setacea BRS Pérola* do Cerrado and *P. alata* BRS *Doce Mel* passion fruit seed oil. Both species come from research studies of Embrapa Cerrado (Brazil), where genetic modification were conducted in order to select species that meets the standard requirements regarding size, skin color, flesh color, number of seeds and a more sweet pulp flavor. In addition the species selected is more resistant to pests typical of passion fruit cultivation in Brazil. To evaluate the oil content in seeds, the lipid determination was carried out by *Soxhlet* method. The seeds were dried at 50 ºC in a convective dryer until the equilibrium moisture was achieved. The crude oil was obtained by crushing in continuous press expeller-type. The pressing processes were made at room temperature at 26 ± 2 °C (cold pressing) or above 50 ºC (hot pressing). For the heating an electric resistance was coupled to the press output. For comparative purposes, passion fruit oil was also obtained using anhydrous ethanol (PA) as solvent. The volatile compounds analysis of passion fruit seed oil was performed by gas chromatography using Solid-Phase Microextraction technique (SPME) in Dynamic Headspace (HS) mode. The chromatograph was attached to mass spectrometer (GC-MS). Identification of the peaks was performed by comparing the mass spectra obtained with those of the device library (Wiley 6th edition) by comparison of the retention index calculated (Kovatz Index) with values reported in the National Institute of Standards and Technology. Discrimination among samples was performed by Principal Components Analysis (PCA) of the absolute chromatographic areas of identified individual compounds obtained by Dynamic Headspace/Solid-Phase Microextraction/Chromatography/Flame Ionization Detector (HS-SPME-GC-FID) using gray fiber Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CA/PDMS). The Unscrambler software version 10.3 (CAMO CORP) was used for PCA. The PCA shows separation among samples obtained applying different pressing temperatures (hot and cold) and by ethanol extraction. The most abundant volatile compounds from *P.setacea* oils were terpens (23 %), esters (23 %), alcohols (19 %), aldehydes and ketons (8 %). Regarding *P. alata* oils, the volatile compounds were in majority esters (35 %), aldehydes (30 %), alcohols (23 %), terpenes and carboxylic acids (9 %). Therefore the volatile profile depends not only on the species, but also on the process used for oil extraction.

1. Introduction

The *Passiflora* genus has a more than 400 species, in which about 120 are native of Brazil (Bernacci, 2003). The Brazil is the world's largest producer of the passion fruit with 900,000 t harvested in 2011 (IBGE, 2013). According to Ferreira (1998), more than 50 species have been cultivated or presented potential for commercialization due to the nutritional quality of their fruits and nutraceuticals properties of their juice, skin and seeds. Nevertheless, commercial crops in the Brazil are based in only one specie, the yellow or sour

passion fruit (*Passiflora edulis*), that represents more than 95 % of orchards, due to the quality of its fruits, vigor, productivity and juice yield (Meletti and Brückner, 2001).
The agro-industrial wastes, in particular those associated with tropical fruits processing, have industrial interest due to their composition rich in lipids, fiber, protein and natural antioxidants.On the other hand, operational costs regarding drying, storage and transportation of these byproducts are still obstacles for economic viability of waste recovery. Nowadays, the waste disposal is restricted by legal norms. Thus, the processing of these materials has been shown to be technically and economically feasible in several productive chains (Lowe and Buckmaster, 1995).
Passion fruit seeds from the juice process are, mainly, used by rural producers in animal feed supplementation, as feed for bovine and poultry, yet with no much adequate technical information, since this volume represents countless tons, adding value to these byproducts is of economic, scientific and technological interest (Ferrari et al, 2004).
The aim of this study was to identify the volatile compounds found in the species *P. setacea* BRS Pérola do Cerrado and *P. alata* BRS Doce Mel passion fruit seed oil. Both species come from research studies of Embrapa Cerrado (Brazil), where genetic modification were carried out in order to select species that meets the standard requirements regarding size, skin color, flesh color, number of seeds, pulp flavor more sweet.
Passion fruit seeds present high oil content in their composition. These oils are composed mainly of oleic and linoleic acids with high nutritional and technological value, vitamins, minerals and bioactive phenolic compounds. These compounds are secondary metabolites present in vegetables which are described as protective substances against oxidative stress (Lima and Pontes, 2011). Traditionally, vegetable oils are used in different sectors of chemical transformation, food, cosmetics and pharmaceutical industries and in particular, passion fruit oil has shown strong potential for these applications (Ferrari et al., 2004; Lopes et al., 2010; Malacrida and Jorge, 2012).
Volatile compounds can be use in different industrial sectors and among their applications may be highlighted their use in food industry for reconstitution and formulation of aroma with more impressive fidelity to natural flavor (Franco, 2003; Nogueira, 2002).
Volatile compounds of passion fruit pulp have been reported by several researches that have identified the presence of esters, ethyl butanoate, ethyl hexanoate, hexyl butanoate and hexyl hexanoate in yellow passion fruit. These four esters constitute about 95 % of total values extracted in the distillation system and simultaneous extraction (Narain et al., 2004). Many volatile compounds were identified in passion fruit and its derivatives (Narain et al., 2004; Vieira, 2006; Macoris et al., 2011). Oliveira et al (2012) identified, by hydrodistillation, about 30 volatile compounds from waste fiber obtained during the pulping of passion fruits. However there are no studies which report the identification and quantification of volatile compounds in passion fruit seed oil. This study revealed that the passion fruit seed oil has volatile compounds that can be classified as aromas of industrial interest and which have the potential to generate natural essences with high added value.

2. Materials and Methods

Raw material: *Passiflora setacea* - BRS Pérola do Cerrado, origin Embrapa Cerrados (Brasília, Distrito Federal) and *Passiflora alata* BRS - Doce Mel*,* origin Rio de Janeiro. To determine the oil content in seeds the lipid determination was performed by *Soxhlet* method (Instituto Adolfo Lutz, 2008).The fruits were pulped and the seeds stored at - 20 °C until their use. After defrosting, the seeds were washed in running water to remove the pulp and mucilage which were still adhered thereto. The seeds were distributed on trays homogeneously and taken to a convective dryer. Drying was conducted at 50 °C in approximately 6 hours until the sample reached its equilibrium moisture (constant weight). These operating conditions were selected in preliminary experiments.
The crude oil obtained was made by crushing in a helical-type continuous press (Expeller), IBG Monforts brand, model CA. The pressing processes were made at room temperature maintained at 26 ± 2 ^{o}C (cold pressing) or above 50 ºC (hot pressing). For the heating an electric resistance coupled to the press output was used. For comparative purposes, passion fruit seed oil was also obtained using anhydrous ethanol (PA) as solvent. In this case, the sample was weighed and distributed on trays to be dried in convective dryer at 70 °C until the equilibrium moisture. After drying, the sample was mixed and homogenized with anhydrous ethyl alcohol (PA). The mixture was stirred at 50 rpm and then heated in a water bath at 55 °C for 2 h. The sample was filtered through filter paper under vacuum. The solvent was removed by evaporation with a stream of air at room temperature.
The oxidative stability of oils was evaluated using a Rancimat equipment (Metrohm model 743) with 3 g of sample under air flow of 20 $L.h^{-1}$ at 110 °C. The induction time was expressed in hours.

2.1 Volatile compound analysis

The volatile compounds analysis of passion fruit oil was performed by gas chromatography using Solid-Phase Microextraction technique (SPME) in Dynamic Headspace (HS) mode. To evaluate the profile of volatile compounds, 1.0 g of sample was heated at 60 °C in 5.0 mL sealed vial for 60 min under stirring. Then, the gray fiber Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) was exposed to the flask headspace to adsorption of substances for another 15 min.

The chromatograph was coupled to mass spectrometer (GC-MS). For semi-quantification, samples were injected into gas chromatograph with Flame Ionization Detector (FID). Both methods are described below:

2.2 Separation and identification by gas chromatography / mass spectrometry (GC-MS)

The analyses for the identification of compounds were performed in cromatograph Agilent model CG-7890. An HP-5MS capillary column (5 % phenylmethylsiloxane and 95 % dimethylpolysiloxane) (30 m x 0.25 mm x 0.25 µm film thickness) and helium/nitrogen/synthetic air as carrier gas was used (1.5 $mL.min^{-1}$). The desorption of analytes occurred at injector temperature of 250 °C for 3 min in splitless mode. The oven was set to 40 °C for 3 min, followed by heating at 40 °C to 240 °C at 3 $°C.min^{-1}$ and remaining at 240 °C for 10 min.

The detection and subsequent obtaining of the absolute areas of the chromatographic peaks for statistical treatment effect was performed using a FID at 280 °C and a mass spectrometry detector (Agilent Technologies model 5975 inert XL/IC MDS with triple-axis detector). The ionization was performed with electron impact at 70 eV, maintaining the transfer line temperature at 280 °C, the ion source at 220 °C and 150 °C in the analyzer. The carrier gas used was helium. Analyses were performed in triplicate.

Identification of the peaks was performed by comparing the mass spectra obtained with those of the device library (Wiley 6th edition) by comparison of the linear retention index (IRL) calculated (Kovatz Index) with values in the literature NIST 1998. For the calculation of Kovats indices, a mixture of n-alkanes standards (C8 to C26) was injected into the GC-MS analysis under the same conditions of the samples.

2.3 Multivariate chemometric analysis of volatile compounds of passion fruit seed oil

Discrimination among samples was performed by principal components analysis (PCA) of the absolute chromatographic areas of identified individual compounds obtained by HS-SPME-GC-FID using gray fiber DVB/CAR/PDMS. The Unscrambler software version 10.3 (CAMO CORP) was used to PCA.

3. Results and Discussion

3.1 Pressing Yield

Table 1 shows the results of lipid content and performance of the pressing process for the two analyzed varieties.

Table 1: *Lipid determination by Soxhlet method, process yield and oxidative stability of oils*

	P. setacea	*P. alata*
Oil content (g/100g)	32.2 ± 1.05	22.5 ± 1.07
Extraction yield (g of oil/ 100 g of dry sample)	30.0	19.0
Pressing efficiency	93.0%	84.4%
Induction time	7.14 ± 0.01	6.15 ± 0.01

The extraction yields were respectively about 30 g /100 g^{-1} for *P.setacea* and 19 g /100 g^{-1} for *P.alata*. This occurred probably due to the more resistent cell wall of *P. Alata* as compared with *P. setacea*. These values were similar to those reported by Homero (2010) who obtained approximately 25 % crude passion fruit seed oil (*P.edulis*). Wilhelm et al (2014) used a press expeller-type, cold pressing and obtained similar results in around 24% for *Passiflora edulis*. The pressing efficiency was high and confirms the potential of obtaining the oil near to the producing region.

3.2 Differentiation among samples

From the chromatograms obtained with the HS-SPME technique we select those with good chromatographic resolution and mass spectra capable of analysiing or identifying by comparison with Wiley library, 6th edition, similarity equal to or higher than 90 %. The broad representation of functional classes, the simplicity, versatility and speed provided by HS-SPME technique with DVB/CA/PDMS fiber, allowed to compare the volatile profile of passion fruit seed oils (*P. setacea* and *P. alata*) subjected to different types of pressing and etanol extraction.

The lower content of aldehydes in the oil *P setacea* (8 %) compared with the oil of *P. alata* (30 %) confirms the results of the oxidative stability of these oils (Table 1). The percentage results observed for the ester of *P. setacea* oil (23 %) and *P. alata* (35 %) were lower than those reported for Narain et al (2004)) and Leão et al (2014) for *P.edulis* species (both 60 %). This probably might be happened because these authors used as the raw crushed material (marc) rich in pulp, while the marc in this work, was subjected to a wash step reducing the volatiles derived from the fruit pulp.

Passiflora setacea

The PCA revealed the separation of the samples with different pressing temperaturas (hot and cold), and those extracted with ethanol (Figure 1A). A total of 53 volatile compounds were identified whose organic functions ranged from terpenes, esters alcohols, aldehydes and ketones. Fourteen of them, the most important ones are shown in Figure 1B.

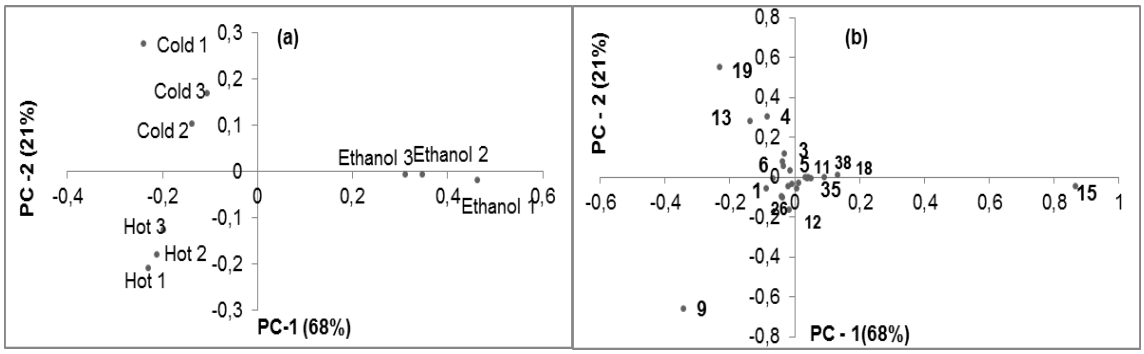

Figure 1*:A: Separation of cold pressing, hot pressing and ethanol extraction; B: Correlation of original scores and variables.*

Figure 1A showed that the arrangement of variables along abscise PC-1 modeled 68 % of the original variables and ordinate PC2 modeled 21 % of the data. The hot pressing processes 1, 2 and 3 are opposed to ethanol extraction processes1, 2 and 3. Extraction with ethanol has a higher weight and contributes to the value of PC1, reflecting greater variability over the identified volatile compounds. In fact this result is consistent because the extraction with ethanol have a greater number of volatile compounds that do not match the pressings cold and hot.

In the graph of scores for key components PC1 and PC2 (Figure 1B), it is observed that the volatile compounds with the same chemical function tend to cluster occupying the same quadrant. Whereas terpenes, volatile compounds that are more predominant in the flavor of the oils, are more at the right of the graph, the other compounds that also contribute to the flavor of oils are spread over the left.

It is observed that the terpene linalool (15) is prevalent among the other compounds identified with a total area percentage of the chromatogram of 25.75 %, ranking the first in order of increasing scores, indicating that the linalool had the highest weight and contributed to the value of PC1.

Table 2*: Correlation among the major compounds identified by the PCA.*

Chemical function	Identification	% Area in process	Increasing order of *scores*
Terpenes	**(18)** alpha terpeniol (terpene alcohol)	4.76	6^{o}
	(13) beta-ocimene (monoterpene)	8.95	4^{o}
	(11) cis ocimene (monoterpene)	2.06	10^{o}
	(38) geraniol (mono terpenoid)	2.02	11^{o}
	(15) linalool (terpene alcohol)	25.75	1st
	(35) linalool oxide (monoterpene)	1.11	14^{o}
Ester	**(6)** hexanoic methyl ester acid	1.99	12^{o}
	(1) ethyl butanoate	3.13	8^{o}
	(19) n-octyl acetate	12.51	2nd
Alcohol	**(5)** 2-heptanol	1.84	13^{o}
	(3)1-hexanol	2.12	9^{o}
Carboxylic acid	**(9)** etil caproate	9.17	3rd
Aldehyde	**(12)** benzene acetaldehyde	3.71	7^{o}
Ketone	**(4)** 2-heptanone	5.59	5^{o}

Passiflora alata

In these case, also the PCA showed a clear separation of the samples with different kinds of pressing (hot and cold) and those extracted with ethanol (Figure 2A) and a total of 20 volatile compounds were identified and plotted (Figure 2B). The organic functions varied among aldehydes, ketones, alcohols and terpenes.

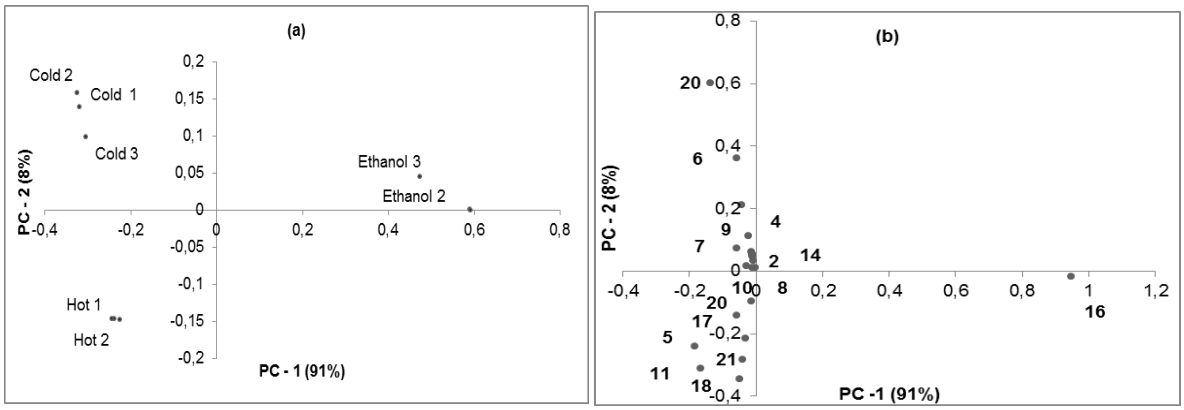

Figure 2:*A: Separation of cold pressing, hot pressing and ethanol extraction; B: Correlation of original scores and variables.*

Figure 2A shows that the arrangement of variables along PC1 models 91% of the original variables. The second principal component PC2 models information of only 8%. As observed before for (*P. setacea*) hot pressings oppose the extraction with ethanol. Figure 2B shows that esters and aldehydes, the volatile compounds that contributed most to the formation of *P. alata* flavor oil are more at left of the graph. This could be related to the fact that the volatile compounds obtained in the extraction with ethanol contributed little to the formation of the flavor of this oil. It is observed that phenylacetaldeyde (16), contributed to the higher weight value of PC1 (91 %). This volatile compound is prevalent among others found since its total percentage of process area in chromatogram was 17.61 %, remaining first in order of increasing scores.

Table 3: *Correlation among the compounds identified by the PCA.*

Chemical function	**Identification**	**% Area in process**	**Increasing order of *scores***
Ester	**(12)** 2- butanoic etil ester	13.17	3rd
	(11) 2-butanoic methyl ester acid	10.95	5º
	(20) acetic ethyl ester acid	1.65	12º
	(15) 3-butanoic hydroxy ethyl ester acid	1.12	15º
	(13) 3-butanoic methyl ester acid	4.39	7º
	(8) butyric methyl ester acid	0.22	21º
Aldehyde	**(7)** 2-methyl butanal	4.72	6º
	(6) 3-methyl butanal	16.21	2nd
	(2) acetaldehyde	0.68	20
	(16) phenylacetaldehyde	17.61	1st
	(1) hexanal	1.00	17º
	(4) isobutanal	1.25	13º
Alcohol	**(9)** 3-methyl 1-butanol	2.34	9º
	(10) 2-methyl 1-butanol	0.83	18º
	(19) phenyl ethyl alcohol	0.54	19º
Carboxilic acid	**(5)** acetic acid	12.76	4º
Ether	**(3)** ethyl ether	2.31	10º
Furan	**(14)** 2 (3H) diidrofuranone	1.11	16º
Terpene	**(17)** trans-beta-ocimene	3.75	8º
Hydrocarbon	**(18)** dodecane	1.24	14º

4. Conclusions

Even though the PCA is a technique of pattern recognition and not a classification technique, it could illustrate the relationship between the graphic elements and scores. There were not statistically significant difference ($p<0.05$) between the extracts by pressing (cold and hot) and solvent (ethanol) as we observed the same behavior in extractions for both species. In the volatile compounds identified it was observed that the terpenes are the more abundant volatiles compounds from *P.setacea* oil which give the oil a nice flavor with floral notes. In the case of *P. alata*, which has very low concentrations of these compounds with the predominant flavor fruity notes.

References

Bernacci, L.C., Meletti, L.M.M, Soares-Scott, M.D., Azevedo Filho, J.A.de, Martins, A.L.M., 2003, Variabilidade genética em caracteres morfológicos, agronômicos e citogenéticos de populações de maracujazeiro-doce (*Passiflora alata Curtis*), Rev. Bras. Frutic. Jaboticabal, São Paulo, v.25, p.275-278.

Ferrari, R.A., Colussi, F., Ayub, R., 2004, Caracterização de subprodutos da industrialização do maracujá – aproveitamento de sementes, Rev. Bras. Frutic., Jaboticabal, v.26, n.1, p.101-107.

Ferreira, F.R, Germoplasma de maracujá, 1998, In: Reunião técnica sobre a pesquisa do maracujazeiro no Brasil, Embrapa, CNPMF, p.48-53.

Franco, M. R. B., 2003, Aroma e sabor de alimentos: temas atuais, São Paulo, Ed. Varela.

Freitas, S.P., Lago, R.C.A., 2007, Equilibrium data for the extraction of coffee and sunflower oils with ethanol, Braz. J. Food Technol., Campinas, v.10, n.3, p.220-224.

Homero, V., 2011, Fábrica recém inaugurada produz óleo natural de maracujá, FAPERJ,<www.faperj.br>accessed 25.10.2011.

Instituto Adolfo Lutz, 2008, Métodos físicos-químicos para análise de alimentos, São Paulo, Ed. 4ª, p.1020.

Instituto Brasileiro de Geografia e Estatística (IBGE), 2013, Produção Agrícola Municipal – Culturas Temporárias e permanentes, <www.ibge.gov.br>accessed 30.05.2013.

Leão, K.M.M, Sampaio, K. L., Pagani, A.A.C., Silva, M.A.A.P.da, 2014, Odor potency, aroma profile and volatile composition of cold pressed oil from industrial passion food residues, Ind. Crops and Prod., v. 58, p. 280-286.

Lima, C.S., Pontes, F. da S, 2011, Determinação da atividade antioxidante de café e maracujá, Projeto final de curso, Universidade Federal do Rio de Janeiro.

Lopes, R.M., Sevilha, A.C., Faleiro, F.G., da Silva, D.B., Vieira, R.F., Agostini-Costa, T. da S., 2010, Estudo comparativo do perfil de ácidos graxos em semente de passifloras nativas do cerrado brasileiro, Rev. Braz. Frutic., v.32, n.2, p.498-506.

Lowe, E.D.,Buckmaster, D.R., 1995, Dewatering makes big difference in compost strategies, Biocycle, v. 36, p. 78-82.

Macoris, M.S., Janzantti, N.S., Garruti, D. dos S., Monteiro, M., 2011, Volatile compounds from oganic and conventional passion fruit (*Passiflora edulis f. var. flavicarpa Deg*.) pulp, Food S. Technol., v.31, n.2, p.430-435.

Malacrida, C.R., Jorge, N., 2012, Yellow passion fruit seed oil (*Passiflora edulis f. flavicarpa*): physical and chemical characteristics, Braz.Arch., Biol. and Technol., v.55, n.1, p.127-134.

Meletti, L.M.M., Brückner, C.H, 2001, Melhoramento Genético, In: Brückner, C.H., Picanço, M.C., Maracujá: tecnologia de produção, pós-colheita, agroindústria, mercado, Porto Alegre: Cinco Continentes, p.345-385.

Narain, N., Almeida, J N., Galvão, M.S., 2004, Compostos voláteis dos frutos de maracujá *(Passiflora edulis* forma *Flavicarpa*) e de cajá (*Spondias mombin L.)* obtidos pela técnica de headspace dinâmico, Food S. Technol., v.24, n.2, p.212-216.

Nogueira, P. C. L., 2002, Contribuição à química dos compostos voláteis e interações com os organismos receptores, Doctorate Thesis, São Paulo, Univ. Est. de Campinas, 211p.

Oliveira, L.C. de, Santos, J.A.B.dos, Narain, N., Fontes, A. dos Santos, Campos, R. S. S., Souza, T. L., 2012, Caracterização e extração de compostos voláteis de resíduos do processamento de maracujá (*Passiflora edulis* Sims f. *flavicarpa* Degener), Ciência Rural, Santa Maria, v.42, n.12, p.2280-2287.

Vandeginste, B. M. G. D. L., Massart, L. M. C., Buydens, S., De Jong, P. J., Lewi, J., Smeyers-verbeke, J.,1998, Data handling in science and technology, In: Handbook of Chemometrics and Qualimetrics, Part 20B, Amsterdam, Elsevier, p.145–198.

Vieira, M.A.R, 2006, Caracterização dos ácidos graxos das sementes e compostos voláteis dos frutos de espécies do gênero *Passiflora*. Master Dissertation, Faculdade de Ciências Agronômicas, São Paulo.

Wilhem, A.E., Antoniassi, R., Faria-Machado, A.F., Bizzo, H. R., Reis, S.L.R., Cenci, S.A., 2014, Diferentes taxas de alimentação de prensa do tipo *expeller* na eficiência de extração e na qualidade do óleo de semente de maracujá, Ciência Rural, Santa Maria, v.44, n.7, p.1312-1318.