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OPTIMIZATION OF HIGH-CONCENTRATION TRANS-ANETHOLE PRODUCTION THROUGH HYDRODISTILLATION OF STAR ANISE

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Abstract - *Illicium verum* Hook essential oil (EO) is composed mainly of trans-anethole (TA), which has therapeutic potential. The extraction is usually by hydrodistillation, taking long hours, leaving the process costly and product with lower quality. Thus, the present study sought to optimize dehydrated fruit fragmentation by Box-Wilson central composite design and EO extraction and EO quality using a 2^{4-1} fractional factorial design. It is concluded that the fruits must be fragmented to sizes smaller than 425 μm using a knife mill. For hydrodistillation, the condition reported as ideal was granulometry < 0.425 mm, 8% mass, 1 hour and water volume of 200 mL. This process provided an EO yield of 10.2% and high-grade TA (96.6%), requiring shorter time of extraction.
Keywords: Antioxidant; Essential oil; Hydrodistillation; *Illicium*.

INTRODUCTION

Star anise (*Illicium verum* Hook) is an aromatic vegetable cultivated in Asian countries, especially in China and Vietnam (Asif et al., 2016; Wang et al. 2007). Its fruit contains volatile oil, resin, fat, tannin, pectin, and mucilage, whereas its seeds contain little volatile oil, resin, and a large amount of non-volatile oil (Chempakam & Balaji, 2008; Li et al., 2010; Wang et al., 2011). Its aroma is due especially to its high essential oil content, 2.5-3.5% (w/w) in fresh fruit and 8-9% in dried fruit (Wang et al., 2007). The essential oil, which is found in the pericarp but not in the seed, consists mainly of trans-anethole: 70-94% (Asif et al., 2016; Bhadra et al., 2011; Chouksey, Upmanyu, & Pawar, 2013; Wang et al., 2007). This active compound molecular formula, weight, and CAS Number are $C_{10}H_{12}O$, 148.205 g.mol^{-1} and 4180-23-8, respectively (PubChem, 2005). Trans-anethole has been increasingly studied for its antioxidant properties (Wong, Lee, & Nurdiyana, 2014), anticancer properties (Asif et al., 2016) and for its potential use in treating neurodegenerative diseases (Bhadra et al., 2011). The star anise essential oil (EO) may also contain estragole (Howes, Kite & Simmonds, 2009), D-limonene (Chouksey et al., 2013), cis-anethole (Wang et al., 2007; Wang et al., 2011), pinene, β-phellandrene, safrol, farnesol and α-terpineol (Chouksey et al., 2013).

For EO extraction, hydrodistillation is the method most commonly employed. However, yields which vary from 4% (v/w) (Bhadra et al., 2011) to 8.2% (Wang et al., 2007) have been reported in the literature, also needing a long process time, from 2 h (Bhadra et al., 2011) to 5 h (Wang et al., 2007).

The goal of this study is to optimize the extraction of this essential oil from dehydrated star anise fruit, as well as to determine its quality in terms of trans-anethole content.

MATERIALS AND METHODS

Dehydrated star anise fruit from the edible species *Illicium verum Hook*, imported from China and commercialized in the Municipal Market of Curitiba, PR, Brazil, was stored under refrigeration after being

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homogenized and quartered to guarantee reliability, especially in terms of its initial granulometry. The granulometric distribution of 100 g of each sample was measured in triplicate after 10 min of agitation in sieves with mesh sizes of 8, 9, 12, 14, 20, 28, 35, 48 and 100 (BERTEL®). Pre-fragmentation has been reported to increase oil yield from the hydrodistillation process (Cai et al., 2014; Li et al., 2010), and it was carried out by using a domestic blender (Clic'Lav, ARNO®). The initial moisture content of the fruit that had been previously ground (WHO, 2011) in a blender (20 g for 20 s), was determined in triplicate by oven-drying at 105ºC (AOAC, 2000; Shreve, Thiex, & Wolf, 2006). An alternative method using infrared drying was also applied in triplicate using System BG-200 (GEHAKA®) at 60°C and 105°C until constant mass (approximately 15 min). The water activity was evaluated using Aqualab Pre (DECAGON®), also in triplicate.

For the grinding studies, the effect of time (t) and mass (m) were evaluated in duplicate by means of a Box-Wilson central composite design (Table 1).

For preliminarly essential oil extraction tests, dried fruits (50g), both ground (in a knife mill, 20 g of sample for 20 s on high power) and whole, were combined with distilled water (500mL) and heated in a Clevenger apparatus for 180 min using a QUIMIS® heating mantle (Q-321A25, 315 W). After cooling, the oil was centrifuged (10,000 rpm at 5 min = 14,257 xg at 5 min) in a THERMO SCIENTIFIC® Centrifuge (Heraeus Fresco 21) in microtubes (1.5 mL). The lipidic phase was tranfered to another set of microtubes containing approximately 7% (m/v) anhydrous sodium sulfate (CAS 7757-82-6, ANALYTICALS®). This salt is commonly used as an oil drying agent (Zhai et al., 2009). The dehydrated oils were subsequently stored under refrigeration (4 ± 1°C) (Bhadra et al., 2011; Chempakam & Balaji, 2008; Wang et al., 2007), and after stabilization (overnight), another centrifugation was carried out under the same conditions. The dried essential oil was hermetically conditioned in amber vials and stored under refrigeration. As an alternative method, a classical Soxhlet extraction was performed in duplicate using ethyl ether (CAS number 60-29-7, NUCLEAR®) to extract the lipid fraction from 8 g of each sample (crushed and whole) contained in filter paper Qualy 15.0 Ø (J. PROLAB®) and capped with cotton for 5 h at 85°C. Lipid fraction yield was determined after the solvent had evaporated for 60 min at 100°C. The oil extracted by the Soxhlet method received the same treatment and storage described above.

Table 1. Grinding tests for star anise fruit.

No.	t (s)	m (g)
G1	10 (-1)	10.00 (-1)
G2	10 (-1)	20.00 (+1)
G3	20 (+1)	10.00 (-1)
G4	20 (+1)	20.00 (+1)
G5	8[2] (-1.41)	15.00 (0)
G6	22[2] (+1.41)	15.00 (0)
G7	15 (0)	7.93 (-1.41)
G8	15 (0)	22.07 (+1.41)
G C (i)[1]	15 (0)	15.00 (0)

[1]Center points in duplicates, i = 1-2. [2]Approximately.

The effect of the granulometry, mass of the dried fruit, extraction time, and the water volume on the yield of star anise essential oil (EO) extracted by hydrodistillation was assessed using a 2^{4-1} fractional factorial design (Table 2). The levels were established according to previous research and preliminary tests.

For granulometry, a small particle size, ideally between 0.25 mm (60 mesh) and 0.425 mm (40 mesh) has been reported to result in higher star anise EO yields (Cai et al., 2014; Li et al., 2010). Therefore, it was decided to work with 0.43 mm bands for the present study.

Masses between 8 and 16% (m/v) were used (Wang et al., 2007; Zhai et al., 2009). Regarding extraction time, preliminary tests indicated stabilization of the volume of essential oil obtained after 3 hours (data not shown). The volume of distilled water varied from 200 mL (minimum quantity required to fill in the system), to 500 mL (maximum for safe operation). The refractive index was measured using an Abbe refractometer (BIOBRIX®) at 25°C (Garber, Herrlinger, & Ciesielslsi, 1962; Tuan & Ilangantileket, 1997). The response variables were yield on a dry basis (Y_{DB}, in g EO/100 g dry matter) and trans-anethole content (TA, %).

The moisture of the EOs and the TA standard (CAS 4180-23-8, EASTMAN CHEMICAL COMPANY®)

Table 2. Hydrodistillation conditions for star anise essential oil extraction.

Tests	G (mm)	m (%)	t (h)	V (mL)
HD1	< 0.43 (-1)	8 (-1)	1 (-1)	200 (-1)
HD2	> 0.85 (+1)	8 (-1)	1 (-1)	500 (+1)
HD3	< 0.43 (-1)	16 (+1)	1 (-1)	500 (+1)
HD4	> 0.85 (+1)	16 (+1)	1 (-1)	200 (-1)
HD5	< 0.43 (-1)	8 (-1)	3 (+1)	500 (+1)
HD6	> 0.85 (+1)	8 (-1)	3 (+1)	200 (-1)
HD7	< 0.43 (-1)	16 (+1)	3 (+1)	200 (-1)
HD8	> 0.85 (+1)	16 (+1)	3 (+1)	500 (+1)
HD C (i)[1]	0.43 < G < 0.85 (0)	12 (0)	2 (0)	350 (0)

[1]Center points in triplicate, i = 1-3.

was assessed according to the official methodology of the Karl Fischer method (AOAC, 2000). Thermogravimetric analysis (TGA) was performed on 10 μL of EO or TA standard in platinum crucibles (200 μL) in a TGA 4000 (PERKIN ELMER®) in a high purity nitrogen atmosphere (50 mL/min) from 30 to 410°C at a 10°C/min heating rate. Gas Chromatography–Mass Spectrometry (GC-MS; GC-2010 Plus, SHIMADZU®, Japan) was conducted based on Bhadra et al. (2011) employing a capillary column DB5 (30 m x 0.32 mm, 0.25 μm film thickness, J&W Scientific Inc., Folsom, CA, U.S.A.) with cross-linked 5% phenylmethyl-silicone. The mobile phase was also Helium gas, but at a flow rate of 1.2 ml/min. The oven temperature was isothermal at 40 °C for 5 min, then increased at 5 °C/ min until 220 °C. It was kept isothermal for 10 min. For the injection, the temperature was 220 °C, and for the interface, 200 °C. In the present study the injection volume used was 1.0 μL with a split ratio of 1:20. TA in EOs was identified by mass spectral data from the NIST/EPA/NIH virtual library and quantified using the areas and the standard curve. The EO quality obtained by regrinding the previous residue (20 g for 20 s) was assessed. After each milling, the fraction lower than 0.40 mm was removed. The material (16 g) was hydrodistilled under condition HD1 (Table 2) of the optimization. Y_{DB} (g EO/g dm), TA content (%) and the yield purity (Y_{DB}/TA) of the EO were also analyzed for these oils.

The experimental design was implemented in STATISTICA 7® (StatSoft, Inc.). ANOVA and Tukey univariate analyses were applied in the *Action Stat* (Estat Camp) supplement from Excel® (Microsoft Corporation). A 95% significance level was used.

RESULTS AND DISCUSSION

Star anise is composed of 6-8 carpels, each of which measures at least 10 mm (Chempakam & Balaji, 2008; Wang et al., 2011). In this study, more than 99.14% (± 0.14%) of the star anise fruits had particles larger than 2360 μm (detailed data not shown), indicating a good integrity of the commercial samples.

A traditional drying tecnique (oven at 105°C) exhibited a higer moisture content in the ground samples (16.55 ± 0.33%, m/m) than previouly reported: 8-12% (Chempakam & Balaji, 2008), 8.59% (Wang et al., 2007) and 14.9% (Zhai et al., 2009). This difference can be explained by either the biological variation between the specimens or error attributed to the technique. In addition to the traditional technique, moisture was also evaluated by infrared drying as well, at 60 or 105°C.

Although moisture results depend on the technique used (Tukey test, $p>0.05$), low dispersions (standard deviation) were observed. The highest moisture content was observed for the drying oven at 105°C (16.55 ± 0.33%, m/m), followed by infrared drying at 105°C (14.63 ± 0.33%, m/m) and at 60°C (12.98 ± 0.14%, m/m); this can be attributed to the loss of other volatile materials. TG and DTG curves of the EO from crushed (20 g, 20 s) star anise fruits or and the TA standard (Figure 1) reinforced this hypothesis. Mass loss exhibited similar behavior for EO (0.788%) and TA (0.536%) between 40°C and 60°C. Likewise, 13.8% of TA and 15.5% of EO were degraded from 40 to 105°C. In other words, there is a relevant loss of EO components when higher drying temperatures are used. Therefore, the moisture evaluation should be carried out at a low temperature to avoid overestimating the water content (e.g., it should be performed at 60°C using the infrared method).

A moisture value of 12.98% was used for calculating the dry basis oil yield. The TA standard mass loss peak (145.1°C) was very similar to that of the EO (147.7°C). Residual mass was also very similar, 4.1% and 3.8%, repectively: a difference of 7.3%. These results suggest that the EO has a high TA content, which has been described to be around 70-94% (Chouksey et al., 2013; Herrera, 2009).

For most halophilic bacteria and mycotoxigenic asperilli development water activity is between 0.75 and 0.80 (Damodaran, Parkin, & Fennema, 2008; Tapia, Alzamora, & Chirife, 2007). For the ground star anise (20g, 20 s), its value was 0.7853 (±0.0062). Thus, the product requires storage at low temperatures until its final consumption.

Fragmentation of fruit samples by a knife mill was simulated using a blender to maximize essential oil production. The dried star anise fruit is not an isotropic material, as suggested by the cumulative grinding curve (Figure 2, a). There are three distinct fracture susceptibility fractions, one producing a fine fraction (indicated by A), another producing an intermediate fraction (B), and yet another producing a coarse

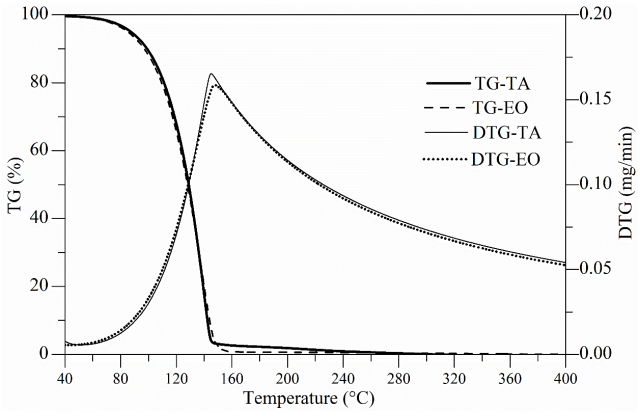

Figure 1. TG (%) and DTG (mg/min) curves for trans-anethole standard (TA) and star anise essential oil (EO).

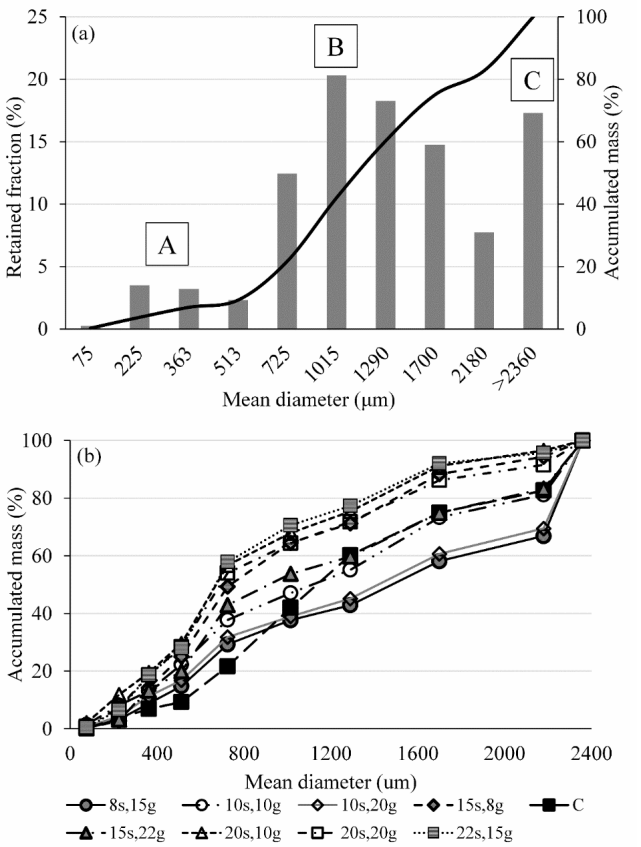

Figure 2. (a) Average profile of the fractions of commercial fruit (15g) fragmentation subjected to milling (15s); (b) Effect of commercial fruit mass and time on accumulated mass on different fractions.

fraction (C). The first may be associated with the more friable portions of the fruit, whereas the latter consists mainly of seeds (Figure 2, a). The seed is not the main source of oil (Chempakam & Balaji, 2008; Li et al., 2010; Wang et al., 2011) therefore, it can be separated to avoid introducing unnecessary material into the hydrodestillation system. There is a significant ($p < 0.050$) linear effect of both time and fruit mass and a quadratic effect of time (Table 3) on the accumulated mass in sieves of different sizes (Figure 2, b). Longer milling times, 20s,10g or 22s,15g, result in larger accumulated fractions in the smaller mean diameter sieves 362.5 μm (19.48% and 18.68%, respectively) and 725 μm (56.76% and 57.87%, respectively) than do shorter milling times 8s,15g and 10s,20g. In these cases, for the same mean diameters, the values were 8.82% and 11.28%, and 29.24% and 31.76%, respectively. Mass distribution within 15 s assumes the intermediate behaviour (Figure 2, b). Such behavior was expected, since the longer the material remains in the grinding equipment, the greater the degree of fineness obtained.

Hydrodistillation is usually more efficient when smaller particles are used, especially those smaller than 60 mesh (250 μm) or 40 mesh (425 μm). Nevertheless, the data were plotted using three granulometry intervals (0-425 μm, 425-850 μm and >850 μm) in order to both evaluate the effects of time and mass and to understand the operation. The effect of time was easily observed (Figure 3, a), but that of mass was not (Figure 3, b). Particles smaller than 425 μm were obtained (Figure 3, c), which is the recommended range for hydrodistillation (Cai et al., 2014; Li et al., 2010). Acording to ANOVA (Table 3), both parameters are statistically significant ($p < 0.050$) and the yield of retained mass (R, %) for particles smaller than 425 μm is described by Equation 1, with actual values:

$$R(t,m) = 0.0623t^2 - 1.1765t - 0.0016m^2 + 16.3733 \quad (1)$$

The condition 8s,15g and the center point (15s, 15g) (Figure 3, c) did not differ statistically between themselves (same letters do not differ significantly by Tukey's test; $p \leq 0.05$), but they were different from the others. At the study limits, the larger times (20 s and 22 s) allowed the use of a wide variation of mass to be crushed. However, the mass to be ground is time dependent (letters b, Tukey's test) with intermediate values. In general, higher milling times increased yield in the 0-425 μm interval and small sample quantities appear to be favorable for the intermediate time (Figure 3, c). A standard sample amount (20 g) and a standard grinding time (20 s) were used in the subsequent oil extraction tests in order to reduce the heating of the samples (thus avoid the consequent loss of volatile compounds) and to avoid crushing the seeds, which according Chempakam & Balaji (2008); Li et al. (2010) and Wang et al. (2011), are poor in trans-anethole.

Different characteristics are observed in oils obtained using different extraction techniques. Hydrodistillation of crushed fruits (Clevenger apparatus) produced an EO with a strong anise flavor, a smooth, transparent, yellowish color, and a viscous behavior at room temperature. It solidified under refrigeration. In contrast, the EO produced by the Soxhlet equipment exhibited a bitter anise flavor with a solvent odor, a dark green coloration, and lower viscosity. These same characteristics were reported by Wang et al. (2007), who produced star anise oil by hydrodistillation and Soxhlet extraction with yields of 8.2 and 9.3%, respectively. In the present study, the yield was lower for hydrodistillation of whole star anise (3.84 ± 0.33 g EO/g dm) and even for the finely ground fraction of the crushed fruit (0-0.425 mm, 6.41 ± 0.50 g EO/g dm). However, crushing had a very important influence on Soxhlet extraction yield (1.20 ± 0.01 g EO/g dm for the whole fruit and 13.02 ± 1.09 g EO/g dm for the crushed fruit). The higher value can be attributed to the concentration step applied by Wang et al. (2007), which was not performed in the present

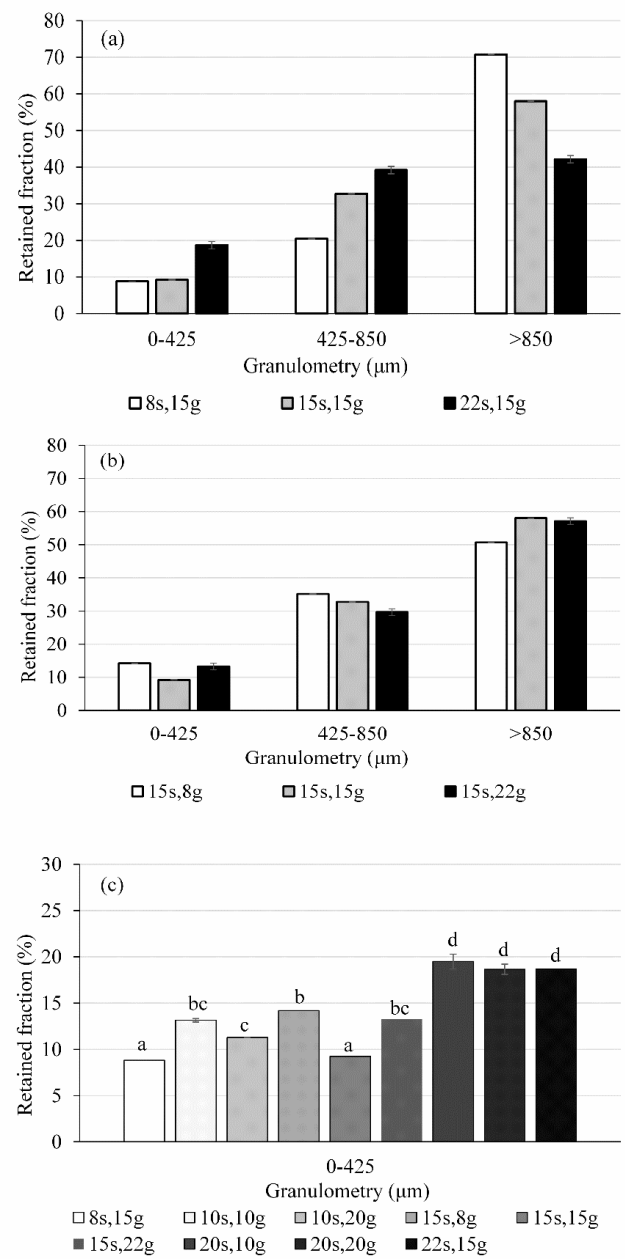

Figure 3. (a) Retained fraction (%) for different time grinding conditions and (b) for 15 s and different mass; (c) Influence of star anise sample mass and milling time on the fine fraction retention (0-425 μm). *Same letters do not differ significantly by Tukey's test (p≤0.05).

study because the physical characteristics of the raw oil obtained would be inappropriate for ingestion (aroma, color, solvent residue).

Moisture content of star anise oil samples submitted to hydrodestillation was very low (0.11 ± 0.01%) and similar to that of standard trans-anethole (0.10 ± 0.01%). Therefore, star anise granulometry, mass, time and initial water volume were investigated for the hydrodistillation technique in order to optimize yield as a function of these variables (Table 4). The highest yield (exp.7, Table 4) was observed for the highest mass ratio between dry fruit and water (16%), the longest operating time (3 h) and using the finest particles (0-0.43 mm). This confirms the importance of reducing fruit size (Cai et al., 2014; Li et al., 2010), and increasing the ratio of fruit mass to water, which should be higher than 10% (Chempakam & Balaji, 2008), in the extraction of essential oil from the matrix. However, only the effect of the granulometry (G) was significant ($p = 0.006 < 0.050$) for the yield, whereas time (t) was marginally significant ($p = 0.050$). Oil Y_{DB} (g EO/100 g dm) can be described by the following model (Equation 2), with actual values:

$$Y_{DB}(G,t) = -9.8960G + 0.2078Gt + 0.7158t + 12.4852 \quad (2)$$

The highest EO yields occurred in experiments HD1, HD5 and HD7 (granulometry G < 0.43 mm), and are higher than the values of 4% and 8.2% previously reported by Bhadra et al. (2011) and Wang et al. (2007), respectively. A Tukey test (α = 0.05) of the refractive index of different EOs indicates that sample HD7 exhibits a significant difference when compared to samples HD2 (p = 0.044), HD4 (p = 0.044), HD6 (p = 0.044) and TA (p = 0.025). TA differed from HD3 (p = 0.041), HD5 (p = 0.031), HD7 (p = 0.025), and HD C (i) (p = 0.034). The parameter was sligthly lower than for standard TA (1.5565) and data reported by other authors for star anise EO: 1.5553 (Tuan & Ilangantileket, 1997) and 1.5585 (Garber et al., 1962) both at 25°C.

Trans-anethole (TA) was the main component quantified in star anise essential oil by GC-MS analysis (Figure 4), in agreement with what has been described in previous studies (Asif et al., 2016; Bhadra

Table 3. Analysis of variance (ANOVA) and effect of time and mass on grinding in order to obtain a fine fraction (< 425μm).

Factor	SS	df	MS	F	p	Effect
(1) t (L)	95.6097	1	95.60974	50.71629	**0.002055***	+6.9141*
t (Q)	34.2640	1	34.26405	18.17540	**0.013020***	+5.4755*
(2) m (L)	2.0118	1	2.01179	1.06715	0.359953	-1.0029
m (Q)	33.7253	1	33.72529	17.88962	**0.013374***	**-1.0330***
1L by 2L	0.2644	1	0.26437	0.14023	0.727057	+5.4323
Error	7.5408	4	1.88519			0.5142
Total SS	153.0203	9				

* Statistically significant (p<0.05).

Table 4. Optimization of EO extraction and selectivity for trans-anethole yield.

Exp.	G (mm)	m (%)	t (h)	V (mL)	Refractive Index	Y_{DB} (g EO/100 g dm)	TA (%)	Y_{DB}TA (g TA/ 100 g dm)
HD1	< 0.43	8	1	200	1.5545	10.2	96.6	9.85
HD2	> 0.85	8	1	500	1.5555	5.4	82.2	4.44
HD3	< 0.43	16	1	500	1.5534	8.0	85.5	6.84
HD4	> 0.85	16	1	200	1.5555	5.1	85.9	4.38
HD5	< 0.43	8	3	500	1.5529	10.6	82.2	8.71
HD6	> 0.85	8	3	200	1.5555	6.7	81.3	5.45
HD7	< 0.43	16	3	200	1.5525	10.8	80.4	8.68
HD8	> 0.85	16	3	500	1.5539	7.3	80.8	5.90
HD C (1)	0.43 < G < 0.85	12	2	350	1.5537	8.9	80.0	7.09
HD C (2)	0.43 < G < 0.85	12	2	350	1.5539	8.9	78.7	6.99
HD C (3)	0.43 < G < 0.85	12	2	350	1.5535	8.9	79.4	7.03

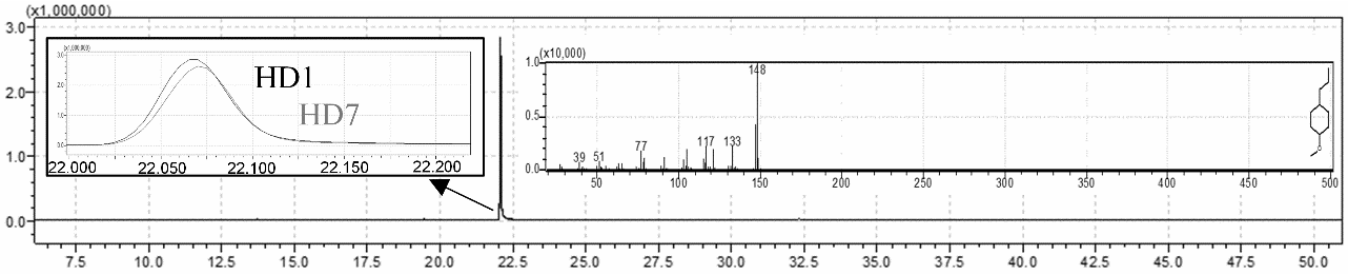

Figure 4. Gas chromatogram of the essential oil in hydrodistillation conditions one (HD1) and seven (HD7). In the zoom, on the left: HD1 and HD7. On the right: EO MS spectra.

et al., 2011; Chouksey et al., 2013; Howes, Kite & Simmonds, 2009; Wang et al., 2007; Zhai et al., 2009). The inset zoom (Figure 4) emphasizes conditions one (HD1) and seven (HD7) of hydrodistillation, since they yielded higher TA content and oil mass, respectively. In the present study traces were also identified of two compounds reported by many other authors: estragole (Chouksey et al., 2013; Howes, Kite & Simmonds, 2009; Wang et al., 2011; Wong et al., 2014) and D-limonene (Chouksey et al., 2013; Zhai et al., 2009). Foeniculin, another substance which has been reported in the edible species of star anise (*e.g.*, *I. verum*) (Chouksey et al., 2013; Howes, Kite & Simmonds, 2009) was also found. The presence of this substance indicates that the samples used in the present study are edible, because toxic species (*e.g.*, *I. anisatum*) do not contain this molecule (Howes, Kite & Simmonds, 2009). Pinene, β-phellandrene, safrol, farnesol and α-terpineol were not identified (Chouksey et al., 2013).

For TA (%) purposes (Table 4), an ANOVA test for the studied parameters indicated no significant effects ($p < 0.050$), but HD1 exhibited the highest TA grade (96.6%).

The yield purity (Y_{DB}/TA) of the EO was highly affected by granulometry ($p = 0.0002$), thus confirming its dependence on the anisotropic portion of the vegetable. Time (t, $p = 0.017$), mass (m, $p = 0.030$) and volume (V, $p = 0.033$) are statiscally significant ($p < 0.050$), but lower than the linear interactions GV ($p = 0.013$) and Gm ($p = 0.014$), which appear to be marginally significant. Therefore, although condition HD7 exhibited a higher EO yield, the best conditions for obtaining an oil rich in TA are those of experiment HD1: a size smaller than 0.43 mm, 8% dried crushed fruit, 200 mL water, and 1 h extraction time. It is assumed that a longer extraction time promotes greater degradation of the bioactive compound of interest.

A successive milling (Mi, i = 1-5) operation (Table 5), with fractions designed to undergo hydrodistillation according to condition HD1 (Table 2), affects the EO yield and TA.

According to a Tukey test ($\alpha = 5\%$), the first grinding differed from the other crushing in these two parameters, generating an oil with a higher grade of purity with reference to the compound of interest. There was a reduction in yield (g EO/g dm), refractive index, and TA (%) when grinding was repeated, reaching 1.4 ± 0.6%, 1.5520 and 45.5 ± 0.9%, respectively. This may prove the supposition that successive milling results not only in the crushing of the fruit, but also in the breaking up of star anise seeds, which contain little

Table 5. Characterization of EO obtained from samples submitted to different millings.

Essay	Y_{DB} (g EO/g dm)*	Refractive Index	TA (%)*
M1	9.5 ± 0.2^{a}	1.5539	96.0 ± 0.5^{a}
M2	5.1 ± 0.4^{b}	1.5535	72.8 ± 4.5^{b}
M3	3.8 ± 0.5^{bc}	1.5535	49.8 ± 1.6^{c}
M4	3.7 ± 0.4^{bc}	1.5535	48.8 ± 1.4^{c}
M5	1.4 ± 0.6^{c}	1.5520	45.5 ± 0.9^{c}

* Tukey test. Equal letters in the same column indicate no significant difference.

volatile oil and hinder both EO yield and TA purity (Chempakam & Balaji, 2008; Li et al., 2010; Wang et al., 2011).

CONCLUSIONS

Analysis of the moisture content of dry star anise fruit should be performed using an infrared analyzer at 60°C to avoid false positive results due to the loss of volatile material. The fragmentation of the dry fruit with a knife mill, used in conjunction with separation by sieving may be an efficient technique for selecting the fraction with the highest essential oil content. The milling of the less friable fractions for hydrodistillation does not increase oil yield, and actually decreases the relative purity of the product with reference to its anethole content. The oil yield from hydrodistillation is positively affected by the use of smaller particles (<425 μm), high particle ratio to water (16%), and longer hydrodistillation times. However, anethole purity is higher after the first moments of hydrodistillation (60 min, 10.2% oil on a dry basis, with 96.6% TA content in the EO). Therefore, collecting the early fraction is a method for selectively producing trans-anethole. The response surface methodology to determine the ideal condition of hydrodistillation of ground star anise gave an equation that is a function of granulometry and time, as well as their interaction. The refractive index confirms the vegetable oil type, but only mass-coupled chromatography was able to characterize its composition.

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NOMENCLATURE

EO	Star anise (*Illicium verum* Hook) essential oil
G (i)	Grinding tests for star anise fruit
GC-MS	Gas Chromatography-Mass Spectrometry
HD (i)	Hydrodistillation condition from fractional factorial design
M (i)	Star anise fruit different millings
R	Retained fraction (%) for different grinding time conditions
TA	Trans-anethole
TGA	Thermogravimetric analysis
Y_{DB}	Tield on a dry basis (Y_{DB}, in g EO/ 100 g dry matter)

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