CHEMICAL COMPOSITION OF CLOVE (*Eugenia caryophyllus*) EXTRACTS AND ITS USE TO INCREASE SHELF-LIFE OF REFINED SOYBEAN OIL

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ABSTRACT – Clove is a spice that presents strong antioxidant potential and could be used as an alternative to the synthetic antioxidants that might present toxicity risks. Objectives were to chemically characterize the extracts of clove bud and to apply it in the soybean oil, as a substitute to synthetic antioxidants. Clove bud extraction was performed using supercritical extraction, hydro-distillation and ultrasound, in order to compare the efficiency of the different extracts in preventing soybean oil oxidation. Identification and quantification of compounds present in clove extracts were performed by GC analysis and the main identified compound was eugenol. Refined soybean oil quality and its shelf-life increase with the addition of clove extracts were evaluate using acid index and moisture determination. Results showed that the use of clove extracts is effective in preventing the increase of acidity in soybean oil for seven days in oven at 60ºC.

1. INTRODUCTION

Clove (*Eugenia caryophyllus*) is a plant widely used for various applications, like flavoring food products and cosmetics, due to its antimicrobial, antioxidant and anesthetic activities. Essential oil of clove has in the composition three phenolic compounds were considered majority, highlighting the eugenol, eugenil acetate and α-humulene (Scherer et al., 2009).

The antioxidant activity of the phenolic compounds is mainly due to their reducing properties and chemical structures that play an important role in the neutralization or scavenge of free radicals and the chelation of transition metals by acting on both the initiation and the propagation step of the oxidative process (Stieven et al., 2009).

The oxidation of lipids is one of the main reactions of food spoilage and implies the emergence of flavors and odors (known as rancidity). This reaction causes reduction of deterioration in nutritional value, as a result of loss of essential fatty acids, and limits the shelf life of many foods. Substances capable of inhibiting or retarding the oxidation are usually added to oils, fats and fatty foods and these antioxidants are eventually able to prolong the shelf-life of foods (Andreo et al., 2006).

Therefore, the aim of the present work was to chemically characterize the extracts of clove bud (*Eugenia caryophyllus*) by gas chromatography and to apply it in the soybean oil, as a substitute to synthetic antioxidants.

2. MATERIALS AND METHODS

2.1 Soybean Oils

The soybean oils, Leve® and Cocamar®, used for the experiments of oil quality, were bought in a local market in Florianópolis/SC, Brazil. The Leve® soybean oil has no synthetic antioxidants, while Cocamar® soybean oil has the synthetic antioxidant TBHQ (tertiary butyl hydroquinone) in its composition.

2.2 Clove Extracts

The supercritical fluid extraction (SFE) of clove was performed in a dynamic extraction unit with CO_2 as solvent, 99.9% pure (White Martins, Brazil). It was performed in a packed bed of 15g of milled material at 100 bar and 50°C with a minimum mass flow rate of CO_2 of 0.2 kg/h for 90 min. The extract was collected in amber flasks and weighted in an analytical balance (AY220, Shimadzu do Brasil Ltda., São Paulo/SP, Brazil) (Sá et al., 2014)

The ultrasound extractions (UE) were run using 7g of clove sample and 210 mL of solvent, placed inside an amber flask. The extraction time was 1h, conducted at room temperature and performed in duplicate. The equipment used was an ultrasonic cleaner bath (Unique Ultracleaner, USC-700) and the UE was performed with ethanol (EtOH) and distillated water (WT) (Sá et al., 2014).

The hydro-distillation (HD) extraction was carried out with 50g of sample and 700 mL of distillated water placed inside a modified Clevenger apparatus for 6h at water boiling point (Sá et al., 2014).

2.3 Chemical Profile

The identification and relative quantification of the compounds present in the clove extract, obtained by supercritical fluid extraction and low pressure extraction (LPE – UE and HD), and in the commercial clove oil were achieved by gas chromatography (GC).

All the SFE and LPE clove extracts were evaluated, except the UE with water because the solubilization of this extract in dichloromethane was not possible. The analysis was performed in a gas chromatograph (GC-2010 Shimadzu do Brasil Ltda., São Paulo/SP, Brazil) and silica column (RTX®-WAX Crossbond® Carbowax® polyethyleneglycol – 30 m x 0,25 mm, 0,25µm).

The samples were dissolved in dichloromethane and injected for analysis. The injection mode was split, injector and detector temperature was 250°C. The carrier gas was nitrogen at a flow rate of 40 mL/min and the amount of sample injected was 1 µL. The initial column temperature was 40°C, held for 8 minutes, then the column was heated at rate of 10°C/min to

150°C, this temperature was maintained for 5 minutes, and the column heated again at a rate of 10°C/min up to 220°C, maintained for 5 minutes. The major compounds of cinnamon were identified using commercial oil (Ferquima®) for comparison.

2.4 Application of Clove Extracts in Soybean Oil

Soybean oil was selected to conduct the study because it is inexpensive and widespread in the food area. The extracts that presented the best results of antioxidant activity (DPPH method, total phenolic content (Folin Ciocalteau) and β-carotene/linoleic acid system) obtained by Sá et al. (2014) were selected to be added to the soybean oil to evaluate the efficiency of these extracts in extending the shelf-life.

The added amounts followed the Brazilian regulations for maximum quantity of food additives in the category of oils and fats (RDC 23 February 15, 2005) presented by the National Health Surveillance Agency (ANVISA) that allows the maximum content of 0,02 g of additive in 100 g of soybean oil.

The synthetic antioxidant BHT (2,6-di-tert-butyl-4-methylphenol) (Sigma-Aldrich, USA) was used as a comparativetothe natural antioxidants in clove extracts.

After the addition of antioxidants, samples of Cocamar® soybean oil were stored in a cabinet simulating the home storage for 18days at room temperature to check the moisture gain of the samples during the period.

The accelerated oxidation test in oven was carried out for samples of Cocamar® and Leve® soybean oil. Four samples of Leve® soybean oil were used. The first sample was defined as control without addition of any additive. The synthetic antioxidant BHT was added to second sample, the third oil sample received the clove extract obtained by ultrasound using ethanol as a solvent, while the fourth sample received the clove extract obtained by hydro-distillation. For Cocamar® soybean oil a fifth sample was studied to evaluate the addition of the clove supercritical extract.

Accelerated oxidation test (Schaal's test): Schaal's test involves heating he sample (50g) at 60°C until the manifestation of the first signs of oxidation. The samples are examined at 7 days intervals during 15 days to evaluate the oxidation state of the product. The Van't Hoff's rule states that an elevation of 10°C in the system temperature doubles the rate of a chemical reaction. Considering the environmental of temperature 20ºC and the Schaal's test temperature of 60°C, there was a 40°C increase. Therefore, a day in oven corresponds to 16 days at room temperature and 7 days correspond to 112 while 15 days correspond to 240 days of home storage at room temperature (Salvador, 2011).

Oil quality test: The analysis of the acid index was performed in samples of soybean oil before and after the accelerated oxidation test to verify the quality of the oil and the increase of shelf-life when clove extract was added. Moisture of the samples stored in the cabinet was evaluated using the Karl Fischer method.

Acid Index: The acid index is defined as the amount in milligrams of potassium hydroxide required to neutralize the free fatty acids in one gram of oil sample (Adolfo Lutz, 2008). The acid index was determined using two grams of oil sample, weighed on an analytical balance (AY220, Shimadzu of Brazil Ltda., SãoPaulo/SP, Brazil) with 25 mL of alcohol-ether solution (2:1) and five drops of phenolphthalein indicator. After mixing the reagents, the mixture was titrated with a solution of 0,01N sodium hydroxide until presented a pink color.

Karl Fischer Method: The Karl Fischer method is extremely accurate to evaluate the water content of a sample. The test procedure involves a chemical reaction between the water and iodine present in the reagent. The iodine is dispensed into the sample in small amounts until the reaction end point is reached. The amount of iodine used for the reaction is directly proportional to the amount of water present in the sample. The analysis of the sample water content was performed using the Karl Fischer equipment (Quimis®, Diadema/SP, Brazil).

2.5StatisticalAnalysis

The oil quality tests results were statistically evaluated by a one-way analysis of variance (ANOVA), using the Software Microsoft Excel 2007 in order to detect significant differences between values in acid index and moisture content of the oil samples. The significant differences ($p < 0.05$) were analyzed by the Tukey's test.

3. RESULTS AND DISCUSSION

3.1Chemical Profile

The relative composition results obtained by CG analysis are presented in Table 1 that shows the name of the identified component and the relative integrated area of the chromatograms for clove extracts obtained by SFE and LPE, and for the commercial oil. The major identified components, in terms of % area peak, were eugenol, eugenil acetate and α-humulene.

The percentages of each compound in the extract have small variations according to the extraction type, but in all the techniques, the major compound is eugenol (about 58%), which is the compound of most interest in this extract. Eugenol reported to be responsible for the fungicide and bactericide effect of clove oil and is much used as an antiseptic (Pereira, 2006).

The chromatograms of commercial clove oil and clove extracts by SFE and LPE are presented in Figure 1, 2, 3 and 4, respectively.

Table 1 – Chemical profile present on clove extract obtained by SFE and LPE, and commercial oil

Compounds	RT (min)(1)	Relative Area %			
		Commercial Oil	SFE	LPE	
				UE-Ethanol	HD
α-humulene	17.8	5.14	15.9	14.6	28.1
Eugenol	27.3	85	58.4	58.5	58.3
Eugenil Acetate	28.7	9.84	25.2	26.9	10.5

(1) RT: retention time.

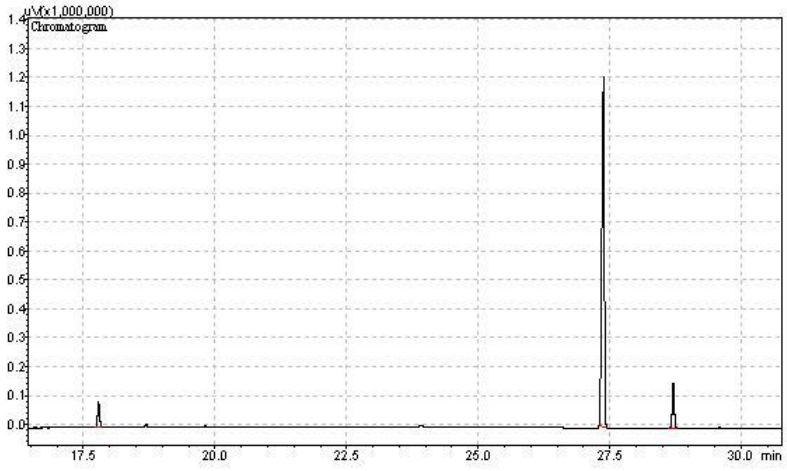

Figure 1 – Chromatogram of commercial clove oil (Ferquima®).

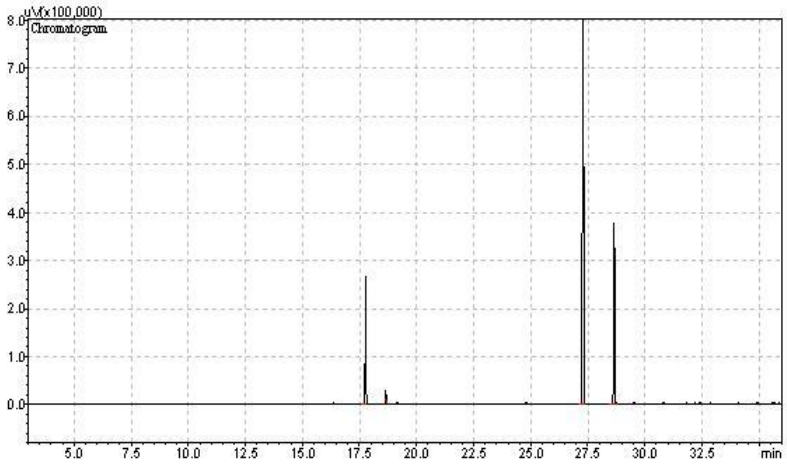

Figure 2 – Chromatogram of supercritical clove extract.

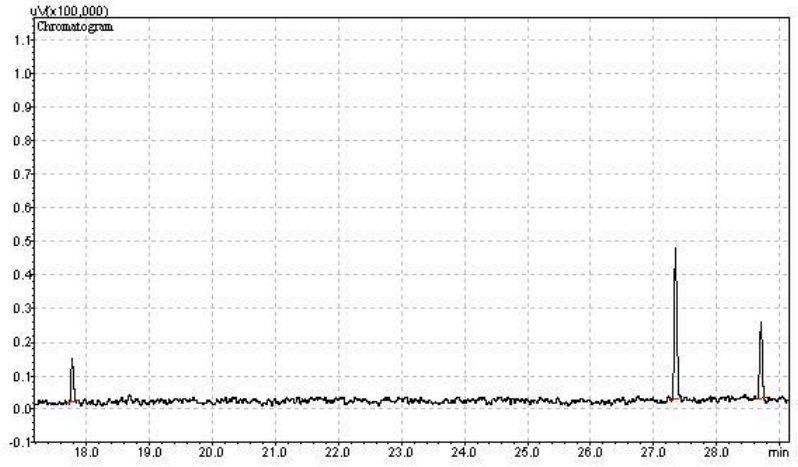

Figure 3 – Chromatogram of ultrasound clove extract.

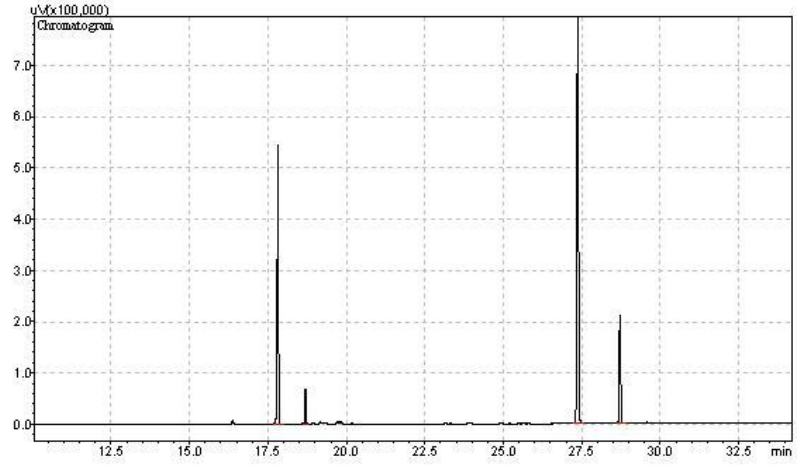

Figure 4 – Chromatogram of hydro-distillation clove extract.

3.1Application of Clove Extracts in Soybean Oil

Acid Index: The results of acid index (mg KOH/g oil) obtained for the samples before the Schaal's test and after 7 and 15 days in the oven at 60°C for the Leve® and Cocamar® soybean oils are shown in Table 2. The method was performed in duplicate and the results were expressed as mean ± standard deviation.

The values for the acid index of Leve® soybean oil before the beginning of the accelerated oxidation test as shown in Table 2 are above the maximum limit allowed by legislation (0.20 mg KOH/g), the soybean oil used in the tests was already out of the quality standards required by legislation according to the acid index (Brazil, 2006).

Table 2 – Acid Index for samples beforetheSchaal'stestandafter7 and 15 days in the oven at60°C for Leve® and Cocamar® soybean oils

Leve® Soybean Oil	**0 days(1) (mg KOH/g)**	**7 days(1) (mg KOH/g)**	**15 days(1) (mg KOH/g)**
Sample Control	$0.21^{a} \pm 0.02$	$0.31^{b} \pm 0.01$	$0.35^{b} \pm 0.01$
BHT	$0.21^{a} \pm 0.02$	$0.25^{c} \pm 0.04$	$0.28^{c} \pm 0.01$
Clove Extract - UE	$0.20^{a} \pm 0.04$	$0.24^{c} \pm 0.05$	$0.32^{b} \pm 0.02$
Clove Extract - HD	$0.21^{a} \pm 0.01$	$0.24^{c} \pm 0.01$	$0.34^{b} \pm 0.01$
Cocamar® Soybean Oil	**0 days(1) (mg KOH/g)**	**7 days(1) (mg KOH/g)**	**15 days(1) (mg KOH/g)**
Sample Control	$0.16^{d} \pm 0.01$	$0.29^{b} \pm 0,01$	$0.36^{e} \pm 0,01$
BHT	$0.16^{d} \pm 0.01$	$0.23^{c} \pm 0,04$	$0.26^{c} \pm 0,01$
Clove Extract - UE	$0.18^{d} \pm 0.02$	$0.22^{a} \pm 0,05$	$0.30^{b} \pm 0,02$
Clove Extract - HD	$0.18^{d} \pm 0.01$	$0.22^{a} \pm 0,01$	$0.31^{b} \pm 0,01$
Clove Extract - SFE	$0.18^{d} \pm 0.01$	$0.24^{c} \pm 0,01$	$0.32^{b} \pm 0,02$

(1) Same letters indicate no significant difference at level of 5% ($p < 0.05$).

According to Table 2, the results of acid index of Cocamar® soybean oil before placing the samples in the oven are below the maximum allowed by legislation.

After seven days in oven, the oils with clove extracts presented less oxidation than soybean oil without additives. The natural antioxidants (clove extracts) were able to retard oxidation in the same proportion as the synthetic antioxidant BHT up to 7 days in the accelerated oxidation test. The 7 days was enough time for all samples, including the ones with BHT, to reach oxidation values above the allowed legislation. Stored in 15 days in the oven, levels of mg KOH/g are above those permitted by law and the oils are not good for consumption. Therefore, to the maximum limit permitted by legislation (0,20 mg KOH/g oil), it is observed that the use of clove oil is effective in preventing the development of acidity in soybean oil.

Karl Fischer Method: The results of the Karl Fischer moisture (% water) for samples of Cocamar® soybean oil stored in a cabinet for 18 days are shown in Table 3. The method was performed in triplicate and the results were expressed as mean ± standard deviation.

Table 3 –Karl Fischer moisture for samples of Cocamar® soybean oil stored in a cabinet for 18 days

Cocamar® Soybean Oil	**0 days(%)(1)**	**18 days (%)(1)**
Sample Control	$0.06^{a} \pm 0.02$	$0.09^{b} \pm 0.01$
BHT	$0.04^{a} \pm 0.02$	$0.088^{b} \pm 0.001$
Clove Extract - UE	$0.047^{a} \pm 0.003$	$0.080^{b} \pm 0.004$
Clove Extract - HD	$0.05^{a} \pm 0.06$	$0.085^{b} \pm 0.004$
Clove Extract - SFE	$0.05^{a} \pm 0.01$	$0.083^{b} \pm 0.005$

(1) Same letters indicate no significant difference at level of 5% ($p < 0.05$).

Results for moisture before placing the samples of soybean oil in the cabinet are below the maximum allowed by legislation (0.1%) (Table 3).

After 18 days, oil samples with clove extract had similar moisture values when compared to soybean oil without additives which shows that the addition of clove extracts in oil has no significant in increasing the oil humidity, according the Karl Fischer method. In18 days, the samples did not exceed the maximum limit allowed by law.

4. CONCLUSIONS

Clove is a spice that presents strong antioxidant potential. The main compound identified by the chemical profile of extracts of clove in terms of percentage of relative area was eugenol (about 58%).

Analysis of soybean oil quality through the methods of acid index for samples stored for 15 days in oven at 60°C (Schaal's Test) showed that the use of natural antioxidants slows oxidation, presenting that the use of clove extracts extends soybean oil shelf-life. Further, sensorial analysis of the oil should be performed to determine the possibility of replacement of the synthetic antioxidant by clove extract natural antioxidant.

The use of clove extract has no significant importance in increasing the oil humidity of soybean oil.

Based in this study, the antioxidant potential of clove extracts obtained by supercritical fluid extraction and ultrasound using ethanol as a solvent is comparable to the efficiency of the synthetic antioxidant BHT up to 7 days in the accelerated oxidation test, showing that it is possible to replace synthetic antioxidants by natural antioxidants.

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