Ultrasound-assisted extraction of clove buds using batch- and flow-reactors: A comparative study on a pilot scale

Ultrasound-Assisted Extraction of Clove Buds with Batch- and Flow-Reactors: a Comparative Study on a Pilot Scale

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Abstract

This study will assess the efficiency of both batch and flow ultrasonic reactors in carrying out the ultrasound-assisted extraction (UAE) of dry clove buds and compare these more recent methods with classic maceration. Flow UAE was carried out in a new multi-horn reactor working in continuous flow mode (450, 900, 1350 mL/min) and at high power density (about 200 W/100 mL). Total phenolic compound content ranged from 190.86 ± 1 to 215.02 ± 3 mg GAE/L extract. The best results were obtained under flow UAE operating at 1350 mL/min (highest phenolic content and best DPPH• free radical scavenging activity). Clove bud volatiles from hydroalcoholic extracts were separated and identified using hyphenated headspace gas chromatography and mass spectrometry. We herein show that the development of large-scale multiple transducer flow reactors operating at high power density enables noteworthy process intensification.

1. Introduction

The design of more efficient extraction processes that may address the requirements of process intensification and energy consumption reduction has been one of the main research topics in recent years. Safety, sustainability, environmental and economic factors are all forcing industry to turn to non-conventional technologies and greener protocols (Chemat, Abert-Vian, & Cravotto 2012). Non-conventional extraction techniques (Cravotto & Cintas, 2007) such as supercritical fluid extraction (SFE), ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) can be applied on pilot and industrial scales and provide worthwhile gains in extraction efficiency and economy.

In many instances, conventional solvent extraction procedures such as maceration, percolation, Soxhlet extraction, extraction under reflux and steam distillation are used for comparison purposes with the aforementioned techniques. Although they are relatively simple methods, they suffer from such disadvantages as long extraction times, relatively high solvent consumption and often unsatisfactory reproducibility (Majors, 1996). Even though non-conventional techniques eliminate most of the above mentioned drawbacks, they may differ in extraction effectiveness. Ultrasound (US) is green as it helps to greatly accelerate extraction process and reduce the total energy consumed. The final extract is more concentrated in soluble material which makes it easier to handle for the formulation and reduce the need for additional process steps. The method is clean, and thanks to the low bulk temperature and the rapid execution, usually it does not degrade the extract. It leaves no residue in the extract and uses no moving mechanical parts inside the extract preventing the occurrence of any pollution. It also offers advantages in terms of productivity, yield and selectivity, improves processing time, enhances quality, reduces chemical and physical hazards and is environmentally friendly (Chemat, Zill-e-Huma, & Muhammed, 2011).

Cloves are one of the most ancient and highly prized spices of the Orient and are widely recognized all over the world for their medicinal and culinary qualities. It is an unopened flower bud that grows on a tree belonging to the Myrtaceae family in evergreen Indonesian rain-forests (*Eugenia* *caryophyllus* (Spreng) Bullock and S. G. Harrison; syn. *Syzygium aromaticum* (L.) Merr). Data in the literature mainly focus on essential oil composition and biological properties.

Eugenol (Fig. 1) is the marker compound in clove essential oil and is of interest as it has been attributed with antioxidant and antimicrobial properties (Bakkali, Averbeck, Averbeck, & Idaomar, 2008). The other main compounds are eugenol acetate and *β*-caryophyllene (Fig. 1). The plant is widely used as herbal drug in the treatment of dyspepsia, acute/chronic gastritis and diarrhea (Geng, Liu, Lv, Yuan, Lin, & Wang, 2007). Eugenol is an important flavoring agent in the cosmetic and food industries (Alqareer, Alyahya, & Andersson, 2006). Several biological properties have also been attributed to *β*-caryophyllene. These include, anti-inflammatory, antibiotic, antioxidant, anticarcinogenic and local anaesthetic activities (Legault & Pichette, 2007). Although hydrodistillation (Santos, Chierice, Alexander, Riga, & Matthews, 2009), supercritical carbon dioxide and superheated water extraction techniques (Clifford, Basile, &·Al-Saidi, 1999; Rovio, Hartonen, Holm, Hiltunen, & Riekkola, 1999; Prado, M. Prado, G., & Meireles 2011; Guan, Li, Yan Tang, & Quan, 2007), have all been applied to the extraction of clove buds, maceration still remains the most common clove bud extraction method on an industrial scale.

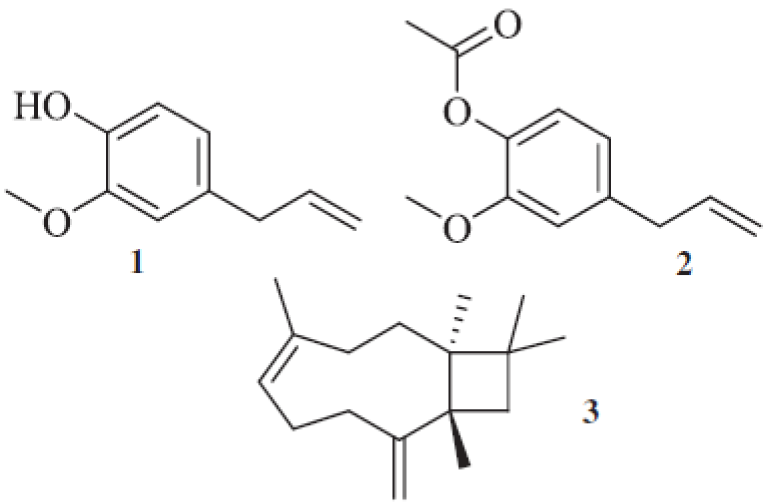

Figure 1 Fig. 1. Chemical structures of the three main compounds in E. caryophyllus (1. Eugenol, 2. Eugenol acetate, 3. β-caryophyllene).

The present work has been undertaken with the aims of investigating the efficiency of UAE performed in batch and in flow modes (Cravotto, Binello, & Orio, 2011) and comparing these techniques with maceration. Despite the existence of flow-US reactor applications in other fields, such as the extraction of bitumen from tar sand and crude oil, residual fuel oil from contaminated soils (Abramov, Abramov, Myasnikov & Mullakaev, 2009) and in food processing (Villamiel, & de Jong, 2000; Zisu, Bhaskaracharya, Kentish, & Ashokkumar 2010) to the best of our knowledge, there are currently no references in the literature that make mention of flow-reactor use for the UAE of vegetal matrices. Our previous experiences in US pilot flow-reactor use have been in waste water treatment (Cravotto, Di Carlo, Curini, Tumiatti & Roggero, 2007) and bio-diesel production (Cintas, Mantegna, Calcio Gaudino & Cravotto, 2010).

2. Materials and methods

2.1 **Materials**

Three batches of dried clove buds of different origin (India, China and Madagascar) were purchased from a local wholesaler. Prior to extraction, all samples were thoroughly milled and mixed to assure powders were homogeneous (microscope measurement 400 μm ±50). The average water content in all samples ranged from 8.5 to 10%. HPLC-grade ethanol and methanol, Milli-Q grade water (Milli-Q Plus system, Milipore, Bedford, USA) were used as solvents. Eugenol (≥ 98%), eugenol acetate (≥ 98%), *β*-caryophyllene (≥98.5%) gallic acid (≥98%), 1,1-Diphenyl-2-picrylhydrazyl (DPPH•) and the Folin-Ciocalteau reagent working standards were purchased from Sigma Aldrich (Milan, Italy).

2.2 **Experimental methods and reactors**

2.2.1. **Maceration (M)**

Ground clove samples (1 kg) were dispersed in 20L of ethanol/water (1:1, v/v) in a stainless steel cylindrical vessel under mechanical stirring (15 min). The suspended material was macerated for 15 h at room temperature as determined ideal in a preliminary investigation. The final mixture was filtered and the solvent was removed under vacuum.

2.2.2 **UAE procedures**

2.2.2.1 **Batch UAE (UAE_b)**

Ground clove samples (1 kg) were dispersed in 20L of ethanol/water (1:1, v/v) under mechanical stirring (15 min) and then subjected to UAE for 45 minutes, using a 25L-US batch reactor (R.E.U.S. - France). The pilot reactor was equipped with a vibrating plate (frequency 25 kHz, effective power 360 W measured in water, power density 18 W/L) mounted onto the bottom of the cylindrical reactor (fig. 2). Effective power was estimated using the classic calorimetric method as reported in the literature (Kimura, Sakamoto, Leveque, Sohmiya, Fujita, Ikeda, & Ando 1996). Sonication was combined with mechanical stirring (60 rpm); the temperature rose from room temperature to 28-30°C after 45 min.

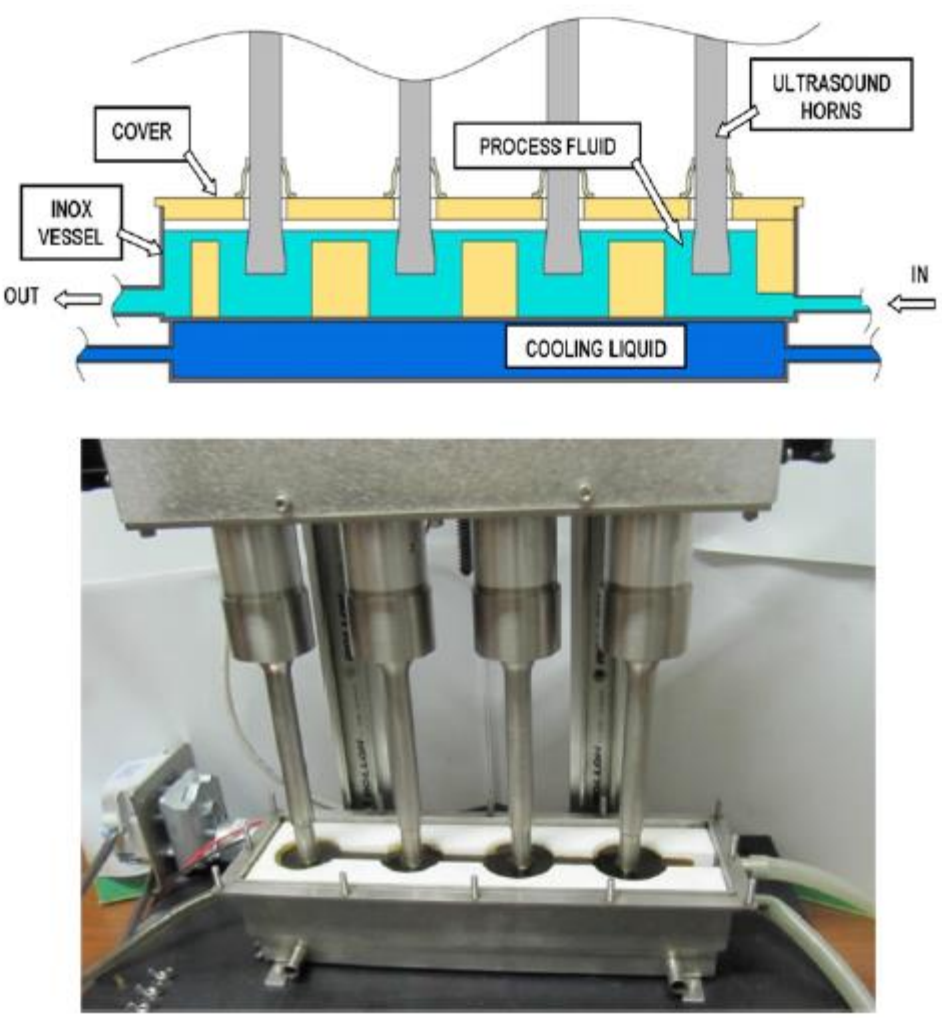

Figure 2. Multi-horn flow reactor.

2.2.2.2 **Flow UAE (UAE_f)**

Ground clove samples (1 kg) were dispersed in 20L of ethanol/water (1:1, v/v) under mechanical stirring (15 min) in a stainless steel cylindrical vessel equipped with a liquid inlet at the top and an outlet valve at the bottom. The suspension was subjected to UAE using a multi-horn flow reactor (four horns, 21.0 kHz) made in collaboration with Danacamerini (Torino, Italy) (Fig. 3 and 3 bis). Particles were maintained in suspension because of the turbulence generated by the circulation at all the three flow rates. The total volume of the four PTFE cavities in which the horns were immersed is 500 mL. A digital generator drives four transducers made up of couples of high-efficiency pre-stressed piezoelectric (PZT) rings (planar PZT Morgan Electronics, diameter of 50 mm) compressed between two ergal blocks. The system enables power to be varied up to a maximum of 1 kW which was monitored by a true reading wattmeter during the experiments. For this extraction three flow cycles (450, 900, 1350 mL/min) were tested by varying the speed of the peristaltic pump (Masterflex L/S Digital Drive, 600 rpm), for an overall time of 45 min. The effective power measured in water was 450 W and the power density 60 kJ/L. The highly regular flow of the pump plays a crucial role for the efficiency of the process.

Overall energy consumption for one cycle, namely one complete passage of the whole suspension volume through the sonication chamber, is a slightly higher in flow procedures (1215 kJ), which also include peristaltic pump consumption, than batch UAE (972 kJ).

2.3 **HS-GC/MS analysis**

Clove bud volatiles from hydroalcoholic extracts were separated and identified using hyphenated headspace gas chromatography and mass spectrometry (HS-GC/MS). Aliquots (10 mL) of the hydroalcholic extracts were placed in 20-mL glass vials with plastic screw caps and Teflon-coated septa. The hydrolcoholic extracts were allowed to equilibrate for 20 min at 70 °C prior to the GC/MS analysis. The headspace device used was an Agilent 7697A provided with a fused silica transfer line coupled with Agilent GC/MS (7820A and 5973 respectively). Flavor extracts were separated using a HP-5 capillary column (30 m x 320 µm x 0.25 µm). Column temperature was initially 40° C, held for

5 min, which was then increased at 5° C/min to 80° C with a final step of 25°C/min to 300°C for 2 min. Helium was used as the carrier gas at 1.2 mL/min. For each analysis, 1 μl was injected at a 1:15 split ratio and injector temperature was 250 °C. The relative percentage areas of every main compound obtained via each extraction technique were compared and expressed as the mean values of three repetitions. The main compounds analyzed were identified by comparing the mass spectra obtained to those of pure standards, where available, and to MS spectra libraries using the match quality index as calculated by the NIST Similarity and Identity Spectrum Search algorithm (NIST 08 and Wiley MS 275).

2.4 **Total phenolic micro-assay**

Phenolic content was determined, according to the method developed by Cicco, Lanorte, Paraggio, Viggiano, & Lattanzio (2009), on the crude extracts obtained both under the maceration and UAE procedures. The proposed method is a variation on the classic Folin-Ciocalteau method as it uses a new combination of time, temperature, alkali and alcohol for the spectrophotometric evaluation of low-concentration phenolic compounds in methanol extracts. The absorption of the final mixtures was measured at 740 nm, in a 1 cm cuvette, on a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). These conditions provided the assay with high accuracy and reproducibility. Quantification was carried out on the basis of a standard curve which was prepared using different dilutions of a 1 mg/mL solution of gallic acid in ethanol as the reference phenolic compound. Total phenolic content is expressed as gallic acid equivalents (GAE, mg/L). All analyses were performed in triplicate and expressed as averages ± standard deviation.

2.5 **Antioxidant activity**

The DPPH· radical scavenging assay was performed according to the method reported by Brand-Williams et al. (1995) with a slight modification. Briefly, 700 μL of a properly diluted sample or ethanol (control solution) were added to the same volume of an ethanolic solution of 100 μM DPPH·.

Mixtures were shaken vigorously and left to stand in the dark at room temperature for 20 min. The absorbance of the mixtures was read at 517 nm. Antiradical activity was expressed as inhibition percentage (I %) and calculated using the following equation:

$$\text{Inhibition (I\%)} = \frac{\text{Abs}_{control} - \text{Abs}_{sample}}{\text{Abs}_{control}} x100$$

Clove extracts have UV absorptions that overlap DPPH· at 517 nm, thus blank solutions (without DPPH·) were prepared to correct any interference that may have been caused by the color.

3. Results and discussion

Three commercial clove batches of different origin (India, China and Madagascar) were extracted and analyzed by headspace gas chromatography coupled with mass spectrometry (HS-GC/MS). Crude extract yield (dry extract/dry plant w/w %), total phenolic compound content and antioxidant activity were determined. The antioxidant activity of phenolic compounds is mainly due to their redox properties which can neutralize free radicals, quench singlet and triplet oxygen and decompose peroxides (Bendec, Vlase, Hanganu, & Oniga, 2012). Generally, the advantages of UAE have been corroborated by a number of studies. Vinatoru (2001a) states that the mechanism of US-assisted extraction consists in the swelling and hydration of plant materials that can subsequently cause enlargement in the pores of the cell wall (Vinatoru, 2001b). UAE is commonly used to facilitate the extraction of intracellular metabolites from plant cell cultures (Kaufman, Cseke, Warber, Duje, & Brielman 1999). The external glands of plant secretory structures can easily be destroyed by sonication, thus facilitating the release of metabolites into the extraction solvent (Vinatoru, 2001c). Ultrasonication produces cavitation phenomena when acoustic power input is sufficiently high to allow multiple microbubbles to be produced at nucleation sites in the fluid. The main factors that affect cell breakage are the water jets and shock waves generated by US. The non-linear portion of US pressure wave dissipates in the form of heat, which makes an effective cooling or a flow system essential. Daghero and Cravotto (2011) developed and patented a sonoreactor operating in continuous mode at high power density with the aim of realizing process intensification in UAE. The same device was applied in the present investigation.

3.1 **Yield of crude extract**

Extraction conditions, technique and reactor type play a crucial role in process efficiency and the chemical profile of the extract. Flow UAE is a novel achievement in the field which exploits a multi-probe system with four titanium horns driven by the same electronic generator. By its geometry, a continuous UAE device can be easily cleaned online in a very efficient and rapid way. We have investigated three different flow rates (450, 900 and 1350 mL/min), each of which was applied during three independent extraction cycles. Figure 4 depicts the percentage yield of the clove bud's crude extracts. The highest yields were obtained using flow UAE at higher flow rates, in contrast, maceration gave the lowest extraction yields. We calculated that the three chosen speeds roughly correspond to one, two and three suspension passages through the sonication chamber. Even though the overall residence time is about the same, a multiple cycle seems to be more effective. Batch reactor UAE was found to be more efficient than the flow reactor operating at low flow rates. Besides the power, the frequency and the geometry of the reactor, this finding highlights the pivotal role of the flow rate in maximizing extraction yield.

The analyses were performed in triplicate and results are expressed as mean values of the percentage yield ± standard deviation (SD).

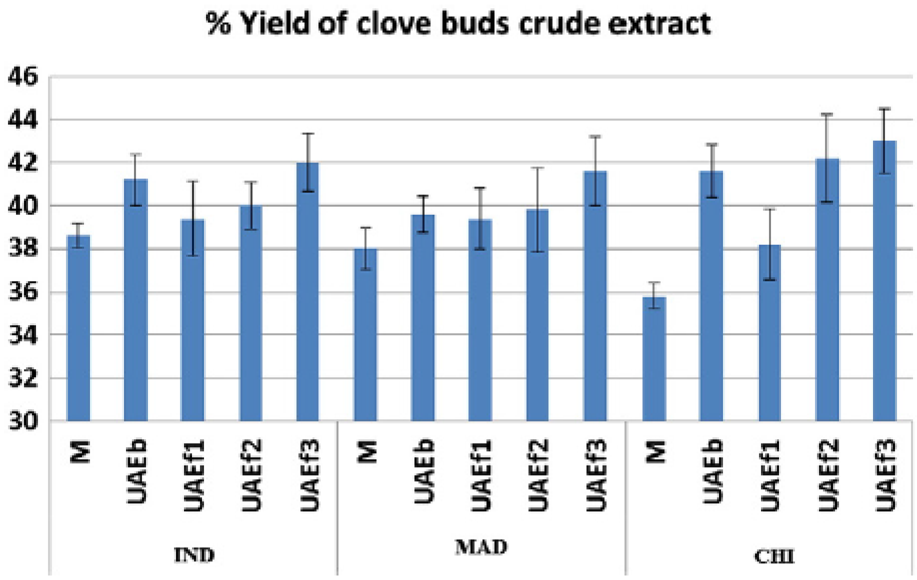

Fig. 3. Total yields of clove buds crude extracts (%, w/w) under different extraction techniques. Yield = weight of the dry extract/weight of the dry plant %. M (maceration); UAEb (ultrasound assisted extraction in batch system); UAEf (ultrasound assisted extraction in flow system: UAEf1 = 450 ml/min; UAEf2 = 900 ml/min); UAEf3 = 1350 ml/min. Sample: IND (Indonesia); MAD (Madagascar); CHI (China).

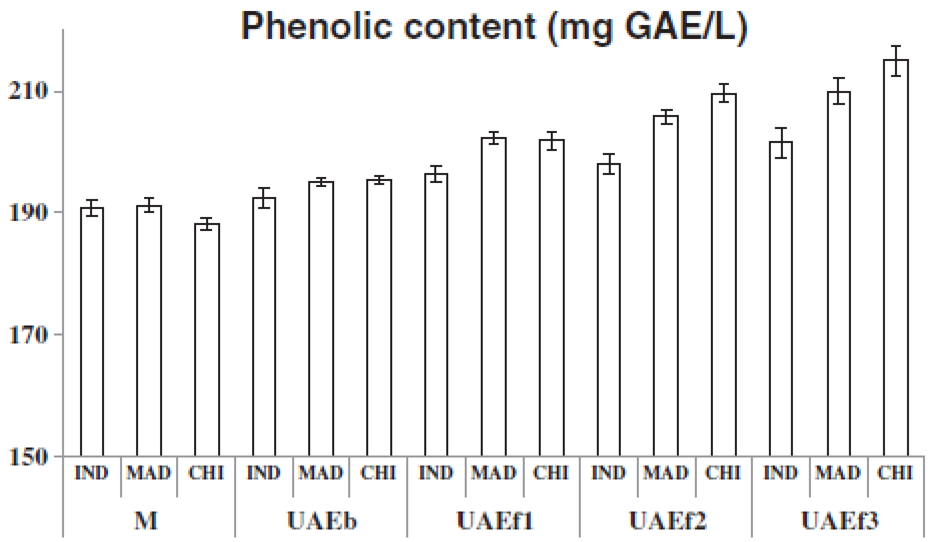

Fig. 4. Total phenolic content of the clove buds crude extracts. M (maceration); UAEb (Ultrasound assisted extraction in batch system); UAEf (ultrasound assisted extraction in flow system: UAEf1 = 450 ml/min; UAEf2 = 900 ml/min); UAEf3 = 1350 ml/min. Sample: IND (Indonesia); MAD (Madagascar); CHI (China).

3.2 **Phenolic content**

Based on our previous studies on the UAE of polyphenols (Casazza, Aliakbarian, Mantegna, Cravotto, & Perego, 2010; Cravotto, Binello, & Orio, 2011), the total phenolic content of the clove bud extracts obtained under different extraction conditions is shown in Table 1. The highest phenolic compound extraction rate was obtained using flow UAE operating at high flow rates. The results shown in Table 1 clearly indicate that, as the sonication flow rate increases, there is a corresponding increase in the total phenolic content of the crude extracts. Total phenolic compound content is expressed as milligrams of gallic acid per liter of extract and ranged from 190.86 ± 1.25 to 215.02 ± 2.5 mg GAE/L extract.

Table 1

3.3 **Antioxidant activity assay**

The DPPH· radical-scavenging activity data of the various clove bud extracts are represented in Table 2. The highest DPPH· free radical scavenging activity was found in the extracts obtained under flow UAE, which once again gave the best performance, providing good correlation with the total phenolic content data. It is worth mentioning that slight differences in the free radical scavenging activity were noticed in the extracts obtained at different UAE flow rates. Antioxidant activity was expressed as inhibition percentage and the experimental results were expressed as the means of three parallel measurements.

Table 2

3.4 **HS-GC/MS analysis**

Volatiles can be released to the headspace at different concentrations depending on their solubility in the matrix, absolute amount and volatility. The trapping analysis of the headspace volatiles is closely related to the sensory profile of the final extract. The area percentages of the principal constituents of the clove bud extracts were monitored in the extracts obtained under the various extraction conditions. The highest main constituent area percentages were found in the extracts obtained using high UAE flow rates (Fig. 5). Typical chromatograms of the clove bud extract headspace flavor volatiles from the three commercial batches are shown in figure 6.

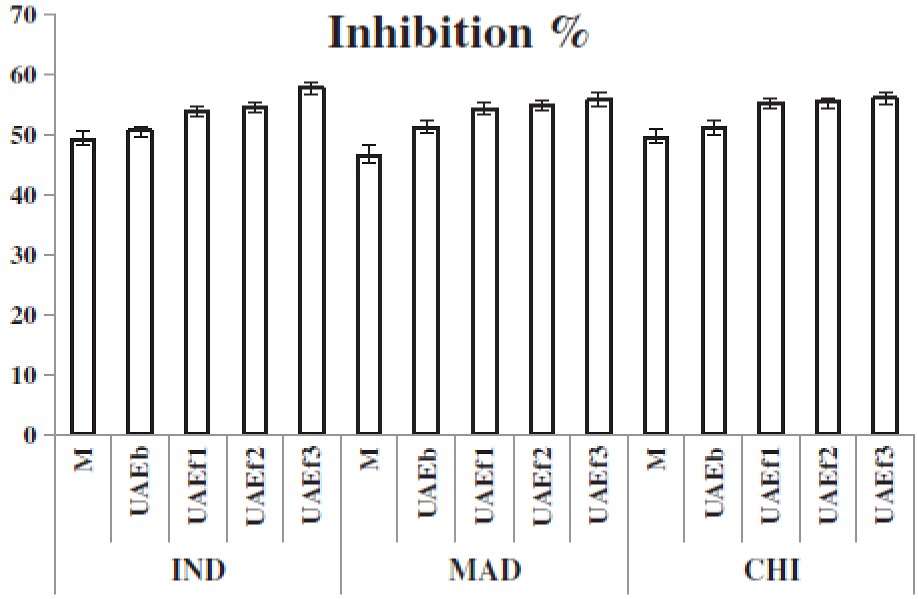

Fig. 5. DPPH-scavenging activity (% inhibition) of clove buds extracts. M (maceration);UAEb (Ultrasound assisted extraction in batch system); UAEf (ultrasound assisted extraction in flow system: UAEf1 = 450 ml/min; UAEf2 = 900 ml/min); UAEf3 = 1350 ml/min. Sample: IND (Indonesia); MAD (Madagascar); CHI (China).

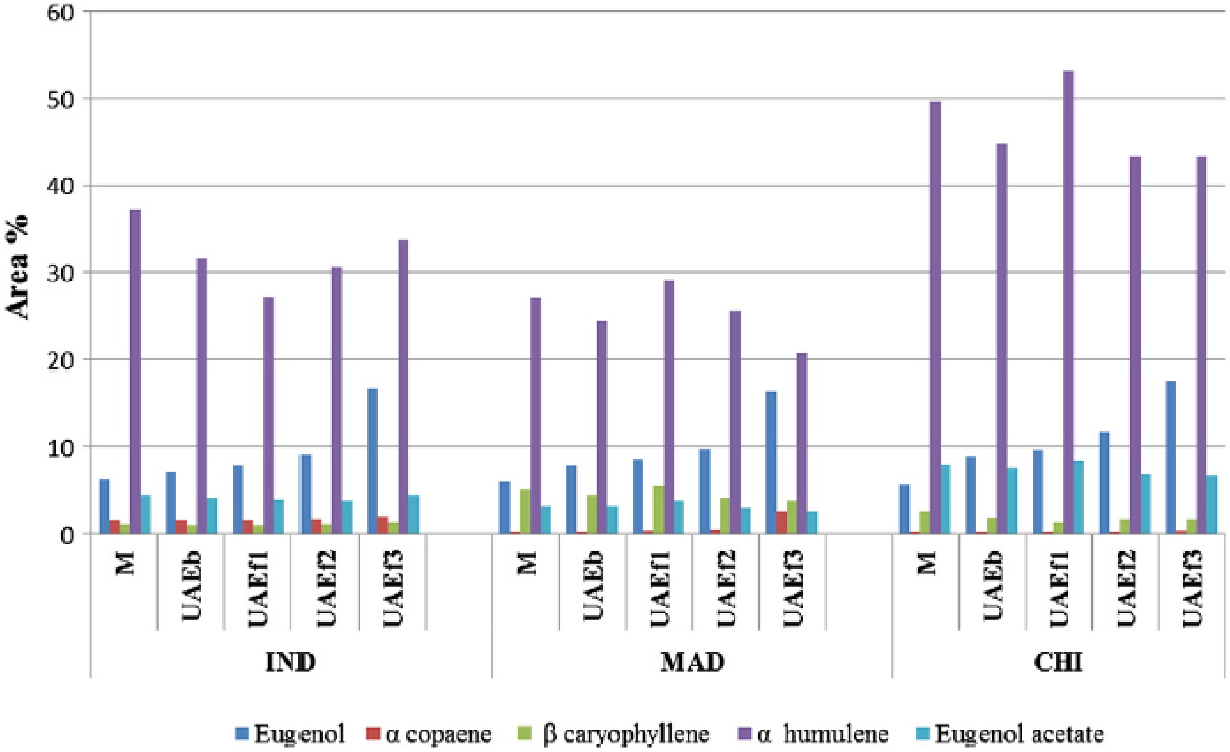

Fig. 6. Relative percentage areas of the main compounds that characterize the aromatic profile of clove buds extracts

Conclusions

The application of flow UAE to process intensification hinges on the development of large-scale multiple transducer reactors operating at high power density. The higher yield and total phenolic content of crude extracts, not to speak of the excellent antioxidant activity, have all proved that dried clove buds can be easily and efficiently extracted using flow UAE.

Acknowledgments

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