Extraction and chemical characterization of the essential oil of *Tagetes pusilla*, in fresh and stored samples

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Extracción y caracterización química del aceite esencial de Tagetes pusilla, en muestras frescas y almacenadas

Extracció i caracterització química de l'oli essencial de Tagetes pusilla, en mostres fresques i emmagatzemades

RECEIVED: 6 MAY 2019; REVISED: 22 MAY 2019; ACCEPTED: 23 MAY 2019

SUMMARY

The objective of the study was to determine the variables for the extraction of the essential oil of *Tagetes Pusilla (Asteraceas),* using water-steam distillation by cohobation and volatile components in fresh and stored samples. Sustained in scientific information, the plant species is collected and classified. The technological process of obtaining essential oil required experimental tests considering factors such as: humidity, volume of water and extraction time at high and low levels and valued by the variable response volume of oil, which allowed to establish a mathematical model for the process. Results showed that the extraction time positively affects the volume of oil obtained. The analysis of the volatile fraction was carried out using gas chromatography / mass spectrophotometer, where the fresh sample revealed 12 components highlighting the anethole and estragole; and the stored sample eight substances highlighting the Trans Anethole, Anisole, p-propenyl and p-Anisaldehyde. Those components confer the aromatic and antioxidant characteristics particular to this essential oil, with research perspectives and immediate application for functional fragrances.

Key words: chemical composition, oil extraction, *Tagetas pusilla*, technological process, volatile fraction.

RESUMEN

El objetivo del estudio consistió en determinar variables para la extracción del aceite esencial de Tagetes pusilla, por destilación agua-vapor por cohobación y componentes volátiles en muestras frescas y almacenadas. Sustentados en información científica se recopila y clasifica la especie vegetal. El proceso tecnológico de obtención de aceite esencial requirió de ensayos experimentales considerando factores como: humedad, volumen de agua y tiempo de extracción a niveles altos y bajos y valorados por la variable respuesta volumen de aceite, lo que permitió establecer un modelo matemático para el proceso. Resultados demostraron que el tiempo de extracción incide positivamente en el volumen de aceite obtenido. El análisis de la fracción volátil se realizó por cromatografía de gases/espectrofotómetro de masas, donde la muestra fresca reveló 12 componentes resaltando el anethole y estragole; y la muestra almacenada ocho sustancias el cuál se resalta el Trans Anethole, Anisole, p-propenyl y p-Anisaldehyde, componentes que confieren la característica aromática y antioxidante particular a este aceite esencial, con perspectivas de investigación y de aplicación inmediata para fragancias funcionales.

Palabras clave: Composición química, Extracción de aceite, *Tagetas pusilla,* Proceso tecnológico, Fracción volátil.

RESUM

L'objectiu de l'estudi va consistir a determinar variables per a l'extracció de l'oli essencial de Tagetes pusilla, per destil·lació aigua-vapor per cohobació i components volàtils en mostres fresques i emmagatzemades. Sustentats en informació científica es reco-

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pila i classifica l'espècie vegetal. El procés tecnològic d'obtenció d'oli essencial va requerir assajos experimentals considerant factors com: humitat, volum d'aigua i temps d'extracció a nivells alts i baixos i valorats per la variable resposta volum d'oli, el que va permetre establir un model matemàtic per al procés. Els resultats van demostrar que el temps d'extracció incideix positivament en el volum d'oli obtingut. L'anàlisi de la fracció volàtil es va realitzar per cromatografia de gasos/espectrofotòmetre de masses, on la mostra fresca va revelar 12 components ressaltant el anetol i estragol; i la mostra emmagatzemada vuit substàncies on es destaca el trans anetol, anisol, p-propenil i 4-anisaldehid, components que confereixen la característica aromàtica i antioxidant particular a aquest oli essencial, amb perspectives d'investigació i d'aplicació immediata per fragàncies funcionals.

Paraules clau: Composició química; extracció d'oli, *Tagetas pusilla,* procés tecnològic, fracció volàtil.

INTRODUCCIÓN

The aromatic and medicinal plants are important in the economy of the villages and are a source of raw materials within the food, cosmetic and pharmaceutical industry, for the production of spices, essential oils and medicines. Essential oils have shown to have useful properties, such as antiplatelet, antithrombotic, anti-inflammatory, antioxidant, antifungal and antimicrobial agents[1]. Nowadays they stand out as the most widely flourishing utilitarian segment of these and other fields especially the perfume and cosmetics industry. In this context, many countries have developed necessary and urgent technological innovations for the processes of intensification and general modernization of the processes used in the manufacture of products derived from aromatic and medicinal plant species.

The genus *Tagetes* (family *Asteraceae*) comprises 151 species described and about 47 are the most accepted[2], and extends through Central America to Argentina. It grows in roads and paddocks or in little inclined spaces free of shrubs and trees. The anise also known as Mexican anise or anise[3], is a very aromatic annual herb with an anise odor and has yellow flowers that give it the characteristic color of the oil. It grows in warm, semi-warm and temperate climates, between 300 and up to 2 400 meters above sea level[4]. In Ecuador, *T. pusilla* or anise, is found as a weed from 1 000 to 3 500 meters above sea level in valleys and inter-Andean hills. However, it adapts to heights less than those indicated. Traditionally in Ecuador, this plant is used in infusions with digestive effects for stomach pain, colic and stomach gas control.

The aerial parts of the plant are used as a food flavoring and gain strength in the food and beverage industry. Its medicinal properties are used in mild dyspeptic conditions used in infusion against catarrhs and gastrointestinal ailments. It acts as a diuretic, antioxidant, antispasmodic, anti-inflammatory, anti-hemorrhagic and relieves cramps and even menstrual cramps, although ingested or rubbing decreases the temperature, chills of the body and removes pain in the ears and head[4,5]. The medicinal use of the product is due, in a great extent, to the antispasmodic, secretolytic, secretomotor and antibacterial effects of its essential oil[6]. It is effective in the fight against enterobacteria and in general has an antimicrobial effect[7]. Also, it is reported that this essential oil of anise grown in hills, presents antibacterial activity of the bactericidal type against *Escherichia coli* and *Salmonella typhimurium*[8].

Volatile oils, essential oils or simply essences, are the natural aromatic substances responsible for the fragrances of flowers and other vegetable organs[9].

Essential oils are compounds made up of several organic substances, which can be alcohols, ketones, ethers, aldehydes, which are produced and stored in the secretory channels of plants. They are usually liquid at room temperature and very volatile. From vegetable material, obtained essential oils are widely used in food, beverages, cosmetics and personal care, and in the chemical and pharmaceutical industries[10].

Tagetes pusilla contains 23,6 of estragole and 70.6 % of Anethole[8]. The LD50 values detected for anethole confer a low toxicity[11], estragol (anethole isomer) colorless aromatic ether is widely used in the preparation of fragrances. Anethole is a typical component of essential oils taken from many aromatic plant species [12]. The anethole or trans-1-methoxy-4-prop-1-enyl benzene or for propenylanilose of the formula (**$C_{10}H_{12}O$**), is colorless, aromatic and fragrance compound, derived from phenylpropane, major component of the essential oil of the drugs cited above. It is largely responsible for its pharmacological activities, among which are those related to the digestive and respiratory system [13].

The widespread use and affordable price of anetol justify the extensive scientific research carried out on the topic to support its use. This review article summarizes the current knowledge of the traditional use of anethole, its pharmacological activities and the possible mechanisms underlying its effects[14]. It has been proven that anethole has muscle relaxant and anticholinesterase, anti-inflammatory and chemopreventive activity[12]. Scientific research provides data on the benefit of daily consumption of food and supplies with antioxidant effect[15]. A dietary antioxidant is a substance that is part of everyday foods and can prevent the adverse effects of reactive species on the normal physiological functions of humans[16]. The anethole known as alilanisol, has larvicidal, insecticidal and antifungal activity and provides an intense and light smell and flavor to sweet anise[17].

The anisole called meovergell known as methoxybenzene, phenoxymethane or phenyl methyl ether and as the anise ether, is an organic compound with the formula **$CH_3OC_6H_5$**. This liquid, insoluble in water and soluble in ether and alcohol, straw-colored to colorless, with a sweet smell of pleasant anisette is used in the food industry, in the manufacture of beer, in the production of fragrances, perfumes, dyes, products, pharmaceuticals, pesticides, pesticides and solvents[18].

Estragol of formula $C_{10}H_{12}O$, 1-allyl-4-methoxybenzene known as tarragon, methyl chavicol, 4-allylanisole, p-allylanisole is colorless, although there may be samples of yellowish color. It has an intense and light aroma of sweet anise and herbaceous smell, but potentially toxic[19]. The aroma of licorice and sweet taste[20]. Estragole is a potential carcinogen, so from an industrial point of view, the manufacture of fennel-containing products requires avoiding optimal conditions using the SFE and ASE techniques to reduce the maximum concentration of estragole in the oleoresin[21]. The insecticide activity is indicated, although further studies are needed.

The essential oil of anisillo, is a transparent, colorless or pale yellow liquid, obtained by steam distillation. Most plants contain 0,01 to 10 % essential oil content. The average amount found in most aromatic plants is around 0,1 to 2 % and the average amount found in most aromatic plants is around 1 to 2 %[22]. For aniseed is close to 1 % and extractions of 3 to 4 hours with 7,53 % trans-anethole[23]. Obtaining essential oil for pampas anise by steam drag achieves yields of 0,2606 % from 1994,98 grams of plant material[24]. Species of *Tagetes filifolia* and *minuta*, yield of 0,24 % to 1,36 % is achieved[25].

Essential oils can be obtained by different methods such as; distillation with water or hydro distillation, distillation by steam drag, distillation with water and steam, distillation with water and steam with application of cohobation. The most frequent is steam extraction, since it is more economical because it uses steam as reagent for extraction of water, uses easy-to-operate equipment and prevents the oil from overheating[26], although there is a lack of appropriate technologies and knowledge to extract them[27].

The method of steam distillation applied to fresh plant materials is viable and economical. However, conventional methods for extracting essential oil are simple, but tend to have low yields[28]. To ensure the extraction process by the method of distillation with water and steam with cohobation is necessary a procedure that supports the flow of steam during extraction. The cohobation system involves the return of the water condensate to the extractor body[26]. The process is carried out at atmospheric pressure, based on Dalton's equation of partial pressures (PA and Pw) considering the oil-water mixture, which form a binary system of two immiscible liquids at a certain temperature where the total pressure (Pt), is equal to the sum of the pressures that each gas exerted at the same temperature (Pt = PA + Pw). For 100 g of dry sample, 700 ml of distilled water is used[29, 30], and for a water-steam distillation 4 liters of water for 3 hours[23].

MATERIAL AND METHODS.

Research and technological development due to its foreseeable repercussions in the processes of transfer and assimilation of technologies are necessary to achieve industrial competitiveness in the immediate future. The study was developed with a multidisciplinary team for the analysis of scientific information on the subject, identification of plants, component analysis, and data validation allowed solid and conclusive results.

The study began with the analysis of the scientific information collected and the collection of *T. pusilla* plants, in climatic floors between 2 000 and 2 500 meters above sea level. The technological process applied to the extraction of essential oil required a stainless steel water-steam distillation equipment with application of cohobation (return of condensed liquid from the water) to the body of the extractor once the oil was released in order to sustain the process of distillation according to figure 1.

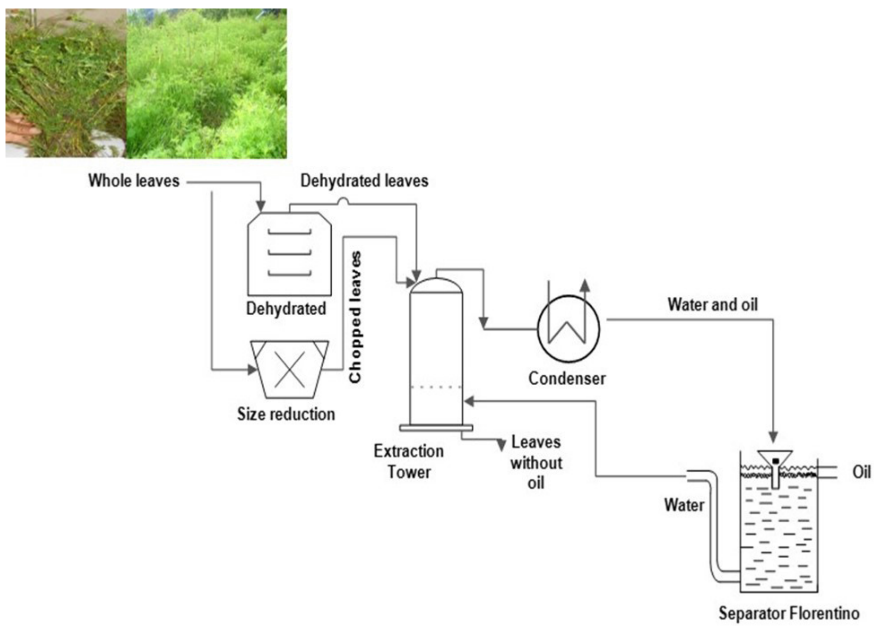


Figure 1. *Technological process of extracting the essential oil using the water-steam distillation method by cohobation.*

Table 1. *Factors, levels and variable response applied to the oil extraction process.*

Factors	Units	Levels		Variable Response
		Medium	High	
Plant moisture (X_1)	%	51,2	67,74	volume of oil (ml)
Volume of water (X_2)	litres	4	5	
Extraction time(X_3)	hours	2,5	3,0	

Table 2. *Volume of oil obtained according to factors and levels of Tagetes pusilla.*

BLOCK	Humidity (%)	Volume of water (litres)	Extraction time (minutes)	Volume of essential oil (ml)
1	-1	-1	-1	9,5
1	1	-1	-1	9,3
1	-1	1	-1	9,5
1	1	1	-1	9,2
1	-1	-1	1	11,9
1	1	-1	1	10,8
1	-1	1	1	11,8
1	1	1	1	11,3
2	-1	-1	-1	9,4
2	1	-1	-1	9,1
2	-1	1	-1	9,1
2	1	1	-1	9,4
2	-1	-1	1	12,1
2	1	-1	1	11,2
2	-1	1	1	12,3
2	1	1	1	11,5

Water volumes were used at levels of 4 and 5 liters, with an extraction time between 2,5 to 3,0 hours. Operation times in the extraction process of essential oil by steam drag are between 1 to 3,5 hours, the knowledge and control of the determining variables of the extraction process of the essential oil ensure the quality of the product[26, 31], the optimization of the technological process applied to the process, productivity and sustainability. The extraction started with two kilograms of fresh plant material with 67,74 % humidity and dehydrated material at a temperature of 50 °C until reaching a 51,2% humidity. In this case we worked with the factors, levels and variable response, according to table 1.

In the extraction process, a factorial experimental design 2^3 was used, with two replications where the response variable is the volume of oil extracted. The results were analyzed statistically with the software Statgraphics plus 4. The identification of the components of the essential oils in fresh and stored sample (Temperature 15-20 °C and Relative Humidity 50-60 %), was carried out by gas chromatography coupled to a mass detector (GC-MS).

RESULTS.

3.1 Process of extraction of essential oil.

Results show that the volume of oil obtained is higher at a high level of extraction time and scarcely perceptible when the humidity of the sample decreases (table 2).

The largest volume of oil is achieved in the first 30 minutes of extraction and the process reaches a yield of 0,52 %. Figures 2, 3 and 4 shows the statistical behavior of the process.

The variance analysis for the extraction of essential oil of *Tagetes pusilla*, responds satisfactorily to regression coefficients for a R-square 99,03 % and adjusted by degrees of freedom (df) to 98,19 % that is more suitable for a confidence level of 9,0 %. The extraction time factor at a higher level is highly significant, consequently it is directly proportional to the volume of oil obtained, while the humidity of the sample is also significant, but to a lesser extent, and inversely proportional to the oil obtained. The moisture-time interaction (CA) reaches significance for a confidence level of 95 %, but for a level of confidence at 1 % it is not significant.

Figure 3 shows that, at a high level of extraction time and low humidity level, a greater volume of essential oil is reached; being the time factor the most significant. The incidence of water volume is scarcely noticeable, because in the process during the extraction time, the water vapor was sufficient due to the application of the cohobation system, consequently the applied technology was appropriate. When evaluating figure 4, the response surface shows that for a certain time the humidity and the water volume slightly affect the volume of oil achieved.

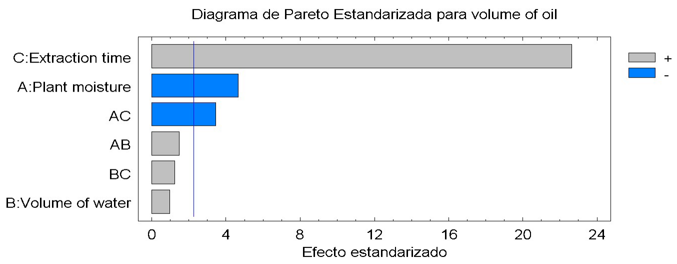


Figure 2. *Pareto diagram for essential oil volume.*

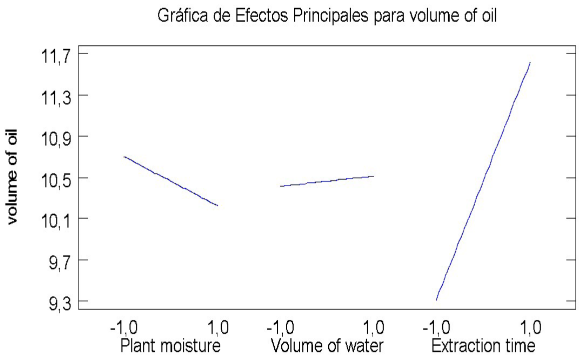


Figure 3. *Graph of main effects for the volume of essential oil.*

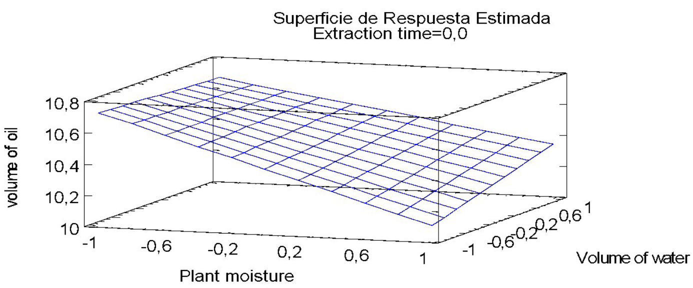


Figure 4. *Estimated response surface.*

The regression coefficient shows the adjusted mathematical model of the essential oil volume of *T. pusilla*, responding satisfactorily to the variable time, a determining factor in the extraction of essential oil from this plant species. Statistically the model indicates an optimum oil value of 12,05 ml. at high level of extraction time and volume of water and at low level of humidity. An adequate combination of factors and levels will allow the optimization and intensification of an extraction process of essential oil of this plant species. The behavior of the equation indicates that the variation of the extraction time variable (X_3) is directly proportional to the volume of oil obtained, as the mathematical equation (1).

$$V_{ac} = 10{,}47 - 0{,}263X_1 + 0{,}063X_2 + 1{,}14X_3 + 0{,}05X_1X_2 - 0{,}15X_1X_3 + 0{,}05X_2X_3 + 0{,}038X_1X_2X_3 \quad (1)$$

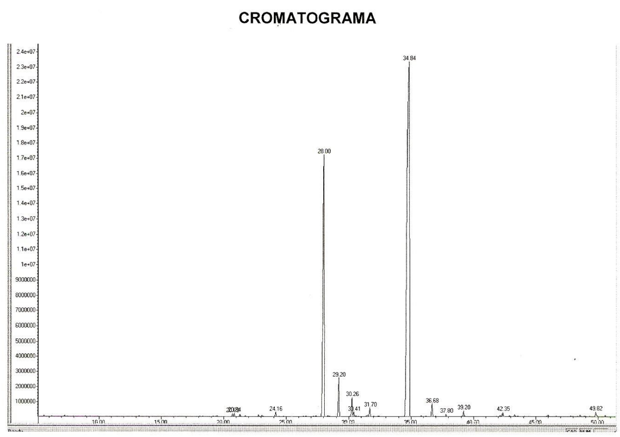
Characterization of the essential oil of *tagetes pusilla*.

The chemical analysis performed by gas chromatography - masses, showed 12 components in the fresh sample, (table 3 and figure 5), in which the Anethole (68,02 %), Estragol (24,52 %) and Germacreno D (2,69 %) stand out, components that give the particular aromatic characteristic to this essential oil. Other studies report the presence of estragole (46,33 %), trans-anethole (31,20 %) and germacrene D (6,37 %)[32].

Table 3. *Chemical composition of the essential oil of the fresh sample.*

N°. component	Holding time (Tr)	Percentage of the compound in the sample (%)	Component
1	20,84	0,20	cipereno
2	24,16	0,28	Trans-cariofileno
3	28,00	24,52	Estragol
4	29,21	2,69	Germacreno D
5	30,26	1,37	Biciclogermacreno
6	30,41	0,30	Beta-bisaboleno
7	31,70	0,65	Alfa-farnesene
8	34,85	68,02	Anethole
9	36,68	0,90	Carvacril acetato
10	39,20	0,38	Unknown
11	42,35	0,27	Metil eugenol éter
12	49,82	0,40	Unknown
TOTAL		99,22	

Figure 5. *Chromatographic profiles of the components of the fresh essential oil.*



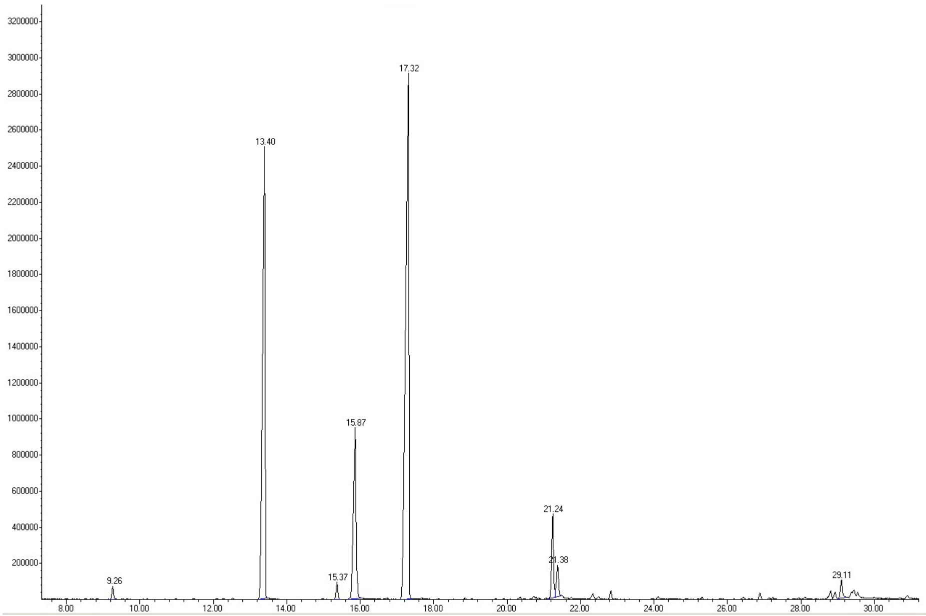
Results of the essential oil after storage at room temperature show a different composition from the percentage of the fresh oil (table 4 and figure 6).

Unlike the composition of the fresh sample, the characterization in the stored sample indicates 8 components, where the Trans anethole (47,22 %), Anisole, p-propenyl- (31,51 %), p-Anisaldehyde (12,59 %), p-Anisylacetato (4,3 %) are the main ones. Trans anethole acts as an antispasmodic and appetite stimulant, analgesic and narcotic, antioxidant and antibacterial [33].

Trans-anethole components stored between 5 and 37 °C for 24 hours, stabilize. At higher temperature higher volatility of the aroma compound is generated.

The actual composition of an essential oil must be demonstrated, in order to limit its variability or volu-

Figure 6. *Chromatographic profiles of the components of the fresh essential oil stored samples.*



bility, and thus be able to demonstrate its true benefits or virtues. The technology applied to intensified processes of extraction of essential oils must be subject to environmental criteria to assess their production and technology applied to the extraction process.

Table 4. *Chemical composition of the essential oil of the stored sample.*

N°. component	Holding time (Tr)	Percentage of the compound in the sample (%)	Component
1	9,26	0,56	L-Linalool
2	13,40	31,51	Anisole, p-propenyl-
3	15,37	0,83	p-Mentha-6,8-dien-2-one
4	15,87	12,59	p-Anisaldehyde
5	17,31	47,22	Trans Anethole
6	21,24	4,3	p-Anisylacetato
7	21,38	1,89	Anisylacetato
8	29,10	1,1	Anisalacetone
TOTAL		100	

CONCLUSIONS.

The application of technologies to processes of extraction of essential oils is developed worldwide in the experimental field, where the application by steam drag according to the water-steam distillation method in a single body by cohobation, is a feasible and friendly alternative for rural sectors that operate with the plant species *tagetes pusilla.*

Many countries generate necessary and urgent technological innovations for the processes of intensification and modernization of the processes used in the manufacture of products derived from aromatic and medicinal plant species in order to allow solid and conclusive results. The characterization of the chemical composition of the essential oil of *tagetes pusilla* allowed the identification of the volatile fraction in fresh and stored sample of the species with very significant percentages in aromatic components, which induce executive actions of immediate application for the industry.

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