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Morpho-anatomical analysis for the quality evaluation in "oregano" commercial samples of Buenos Aires City (Argentina)

[Análisis morfo-anatómico para la evaluación de calidad en muestras comerciales de orégano de la Ciudad de Buenos Aires (Argentina)]

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Abstract: Species of *Origanum* L. (Lamiaceae) are traded in Argentina mainly as flavorings. With the aim of evaluating the quality in "oreganos", a random sampling of twelve trademarks commercialized in Buenos Aires City was performed. The packaging and organoleptic characters of the samples were examined; the components were separated under stereomicroscope and calculated the percentages. Subsequently, a microscopic analysis by obtaining and observing macerations was undertaken. The studies conducted revealed the presence of foreign matter mainly composed of own stems and bran cereal in some samples. The percentages found were higher than those suggested by the Argentinean Food Codex. We conclude that the foreign matter causes a reduction in the plant parts used, introduces adulterants and negatively affects the quality of the product. The methodolgy used in this study contributes to a better control over the "orégano" for trade.

Keywords: quality evaluation, foreign matter, oregano, *Origanum* spp.

Resumen: En Argentina se comercializan especies de *Origanum L.* (Lamiaceae) principalmente como condimentos. Con el objetivo de evaluar la calidad de oréganos se realizó un muestreo sobre doce marcas comercializadas en la Ciudad de Buenos Aires. Se examinaron los envases y caracteres organolépticos de las muestras; se separaron los componentes bajo lupa y se calcularon sus porcentajes. Posteriormente, se realizó un análisis microscópico mediante la obtención y observación de macerados. Los estudios realizados revelaron la presencia de material extraño, constituido mayormente por tallos propios y por salvado de cereal en algunas muestras. Los porcentajes hallados resultaron superiores a los sugeridos por el Código Alimentario Argentino. Se concluye que la materia extraña ocasiona una disminución de las partes usadas, introduce adulterantes e incide negativamente sobre la calidad del producto. La metodología implementada en este estudio contribuye al control del orégano destinado al comercio.

Palabras Clave: evaluación de calidad, materia extraña, orégano, *Origanum spp.*

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INTRODUCTION

The genus *Origanum* L. (Lamiaceae) comprises aromatic plants used as flavorings with the common name "oregano". Following Ietswaart's classification (1980), within the 10 sections of the genus there are 42 species, 6 subspecies, and 17 hybrids recognized (Kokkini, 1997). Hybridization is a common phenomenon in the genus *Origanum* and is expected when two species are growing together in nature. Oreganos are usually sub-shrubs, 30-80 cm high with roundish to slightly quadrate stems. Leaves are entire, oval shaped and frequently pubescent on both surfaces, varying in lenght from 2-40 mm and in width from 2-30 mm. Flowers, arranged in spikes which can form panicles, are protected by hairy, green or purple bracts. They can be leaf-like in texture, color and indumentum or somewhat different. The calyces are pilose, tubiform, with sub-equal triangular teeth or bilabiate with unequal teeth. These are the most variable parts within the genus. Corollas are bilabiate and white, pink or purple. Fruits are smooth, dry, globose and brown nutlets (Ietswaart, 1980; Xifreda, 1983; Xifreda, 2005; Binda *et al*., 2010).

Oregano is one of the aromatic herbs that is known for its worldwide economic importance. Global production is estimated at 60,000 tons per year, being U.S.A, Mexico, Turkey, Greece, Israel and Morocco the main producer countries (SAGPYA, 2005).

In Argentina several species, subspecies and hybrids of this genus are cultivated. The production is estimated at 600 tons per year over an area of 500 hectares. The provinces of Mendoza, Cordoba and San Juan are the most suitable regions for growing and are also the main producers (50 %, 24 % and 12 %, respectively), followed by Salta, Santa Fe, Chubut and Entre Ríos (14 %). The highest yields are obtained in warm temperate to temperate climates (Lenardis *et al.*, 2006). Nationally, in 2010 the main aromatic products exported were coriander, chamomile, oregano and black pepper. These products concentrated 93 % of export volume and oregano represented 7 % of total export volume (CAEMPA, 2005).

According to survey works performed, the most cultivated species are *Origanum vulgare* ssp *viridulum* (Martrin-Donos) Nyman and *Origanum* x *majoricum* Cambessedes (hybrid between *O. vulgare* ssp *vulgare* and *O. majorana*) (Xifreda, 1990; Xifreda, 2005). It is quite common, however, refer to varieties, ecotypes or clones of cultivated oreganos named "compacto", "cordobés", "criollo", "mendocino", "nativo", "verde limón", "negrito", "peruano" and "green Spanish", depending on the growing area and according to their agronomic performance (Di Fabio, 2005; Binda *et al*., 2010; Farías *et al*., 2010; Torres *et al*., 2010; Torres *et al*., 2012).

Although the popular use is as flavorings for foods, *Origanum* species have been used as medicinal herbs with tonic, digestive, vulnerary, hepatic-intestinal, and also antiseptic properties. The most frequent use is as infusion (Rouquaud and Videla, 2000; Roig, 2001; Eyssartier *et al*., 2009). Numerous scientific researches on *Origanum* essential oil composition and properties have been developed in recent years, investigating its antibacterial effects, antifungal activities and antioxidant capacity (Manohar *et al.*, 2001; Amadio *et al*., 2006; Ahmed Chaudhry *et al*., 2007). More recently, the use of the essential oil as a potential food additive was evaluated (Amadio *et al*., 2011).

Other researches referred to genetic marker analysis to differentiate subspecies of *Origanum*, which are characterized by high morphological diversity, and resolve taxonomy problems. It has been demonstrated that those markers are not affected by environmental conditions and could be used for surveying genetic diversity (Azizi *et al*., 2009).

In the Article 1226 of Argentinean Food Codex (CAA), the leaves and flowering tops (flowers and bracts) of orégano are indicated as the parts used (CAA, 1969). According to a Resolution amending Article 1226, a maximum up to 5 % of foreign matter of which, 3 % must correspond to stems of the same plant and 2 % to innocuous material from other plants, is admitted (Resolución Conjunta 48 y 147, 2008). More recently, in the Quality Protocol of Oregano and in order to achieve a premium quality, were established a maximum of 2 % for the stems of the plant and 0.5 % for other plants materials (Resolución 76, 2009).

In a preliminary analysis of oregano commercial samples were detected irregular fragments similar to leaves cut into pieces, which were identified as colored bran cereal (Varela *et al*., 2009). These elements were present in high proportions exceeding the percentage established by the CAA for foreign matter in oregano.

A more detailed survey on a greater number of samples was performed to take into account all the foreign elements that appeared in the commercial products. In this study, different samples of oregano trademarks sold in Buenos Aires City were analyzed in order to achieve the botanical control, to check the purity in terms of plant parts used and detecting,

identifying and quantifying the foreign elements that might affect the quality of the marketed product.

MATERIALS AND METHODS

Materials

Three different samples of twelve (12) trademarks of oregano were analyzed; one of them unpacked, in bulk (A), and 11 sold in packages (B, C, D, E, F, G, H, I, J, K, L). The samples were collected at various shops in the City of Buenos Aires following a simple random sampling. The lower net content packages according to the trademark (15 g, 20 g, 25 g and 30 g) were selected; for that sold in bulk, samples weighing 20 g were taken. All the samples are deposited in *Catedra de Farmacobotanica*, Faculty of Pharmacy and Biochemistry, University of Buenos Aires.

Methods

The plant material was analyzed according to the following protocol:

1) Analysis of packaging, labeling and organoleptic characters: the first two items were carried out in the packaged products and the organoleptic characters (odor and color) in all the samples.

2) Macroscopic analysis: a) macroscopic description; b) determination of foreign matter. The samples were homogenized extending the entire content of the package and mixing well the components. Representative portions 10 % equivalent of the net content were taken by quartering method (WHO, 1998). Those portions were observed with a Carl Zeiss stereomicroscope and the components were described, separated, weighed on a Scout Pro 401 (0.1 g) scales and their percentages were calculated. The separate components were photographed by a Zeiss 47 50 52 stereomicroscope with camera.

3) Microscopic analysis: soft maceration of some components in the samples was made by the caustic alkali method. The material was placed in a beaker with 10 ml of 5 % sodium hydroxide and boiled for 5-10 minutes. Once cooled, the content was filtered and the material retained in the filter was washed several times with water, and then preserved in 70 % ethanol (WHO, 1998). The preparations obtained were observed, described and photographed with a light photomicroscope Zeiss Axiolab MC 80 DX.

4) Statistical treatment of data: a One-way ANOVA, a F-Test and statistical charts representing the data were done through Microsoft Office Excel 2007 software.

RESULTS AND DISCUSSION

Analysis of packaging, labeling and organoleptic characters

The commercial oregano was in polyethylene or polypropylene sealed bags, opaque but with a transparent portion which revealed the contents. Labels were printed on the packaging providing information about net content, packing and expiry dates, batch number, name and commercial address of origin and/or packing, numbers of RNE (National Establishment Register) and RNPA (National Foodstuffs Register) and barcode. Some trademarks did not include the date of packing while others had no batch number. From 36 samples analyzed, 42 % (5 trademarks) had no packing date, 33 % (4 trademarks) lack both packing date and batch number, and 8 % (1 trademark) had no batch number. Only 6 samples (17 %), corresponding to 2 trademarks had the full information. The product sold in bulk was contained in a wooden box with a glass front and an acrylic transparent cover in the top. It had a label with the common name "oregano" and no other information. It is quite evident that very poor information is present in the unpacked samples respect to their origin, packing and expiry dates, if compared with the packed products.

Organoleptic characters indicated an aromatic distinctive odor in all the samples but weaker in some of them, and a dark green color except for a few trademarks that had a fainter color. The product sold in bulk had, in one of the samples, leaves in bad conditions and an excessive amount of stems.

The commercial samples of oregano sold in Buenos Aires City were properly packaged regarding to the plant parts used. However, some deficiencies in the labeling were detected. The hygienic conditions were good in general, as no eggs or larvae, no alive or dead insects or parts of them were detected in any of the samples.

Macroscopic analysis

Characteristics and percentages of all the separate components in relation to the weights of the average samples were represented in Table 1. Genuine elements of oregano and foreign elements, observed under the stereomicroscope, were shown in Figure 1.

a) Macroscopic description: samples were constituted, in variable proportions, by entire leaves and leaves fragments, bracts, flowers with their respective calyces and corollas, and also by stem portions of the same plant. The leaves were dark green, pubescent on both sides and variable in size depending

on the trademark. The bracts, also hairy, had a different texture respect to the leaves and were light green or green with purple hues. Regarding to the flowers, white, pale pink or pink-purple corollas were observed in the different samples and two types of calyces: bilabiate with three long and two shorter teeth, or tubiform with five sub-equal triangular teeth. The plant stems were usually purple (Figure 1A). Every other element with different characteristics from those considered above was classified as foreign matter.

b) Determination of foreign matter: diverse heterogeneous materials as pieces of leaves, stem fragments, spikelets, fruits and seeds of other plants were found. Some samples had soil particles, little stones and abundant dust. In four trademarks was detected a significant amount of flat pieces with irregular shape, variable in size and pale yellowish color with green hues on the edges (Figure 1 B-D).

As a result of the observations, all the commercial samples had the components established by CAA in variable proportions: leaves, flowering tops (bracts and flowers) and stems of the same plant. Taking into account that the majority of the oregano essences comes from the leaves, it is important to notice that several samples had a low percentage of those organs and a high percentage of bracts. Besides, some samples did not contain leaves in a good condition, dark green colored, but brownish or dark.

In all them, the own stems of the plant were present in very high percentages when compared with the other parts, being higher than the 3 % suggested by the CAA.

On the other hand, heterogeneous materials accompanying the samples were considered as foreign matter. The quantitative data showed higher percentages than those established by the CAA for foreign elements. While most of the samples had pieces of stems, leaves, flowers and some fruits or seeds not belonging to oregano, the presence of yellowish-green colored fragments in four trademarks (A, B, C and D) was notorious. Their proportion greatly exceeded the maximum percentage established by the CAA, therefore those elements can be considered as an adulterant. In two of the trademarks (A, D), however, the fragments were not present in all the samples, varying considerably within the same trademark. This could be explained by the little homogeneity in the different batches and by the lack of control during packaging.

The methodology employed for macroscopical analysis in the present work, was similar to that used by other authors in botanical-hygienic quality control of commercial samples used as crude drug (Luján and Barboza, 2008), or for studies about marketing and quality control of medicinal plants (Cuassolo *et al*., 2009). In all of them referred to labeling analysis; presentation, legitimacy and purity of material; foreign matter separation and determination of adulterants.

Microscopic Analysis

Soft macerations were made on the genuine leaves of the samples and on the yellowish flat irregular fragments. Microscopic preparations of the macerated materials were shown in Figure 2.

Microscopic examination revealed that genuine leaves of oregano consisted of: a) polygonal or irregular epidermal cells; b) diacytic stomata; c) two types of non-glandular trichomes: short unicellular and long pluricellular; d) two types of glandular trichomes: capitate with a unicellular head, and peltate with a pluricellular (8-12) head (Figure 2 A-C). On the other hand, the yellowish fragments consisted of: a) tangentially elongated cells with punctuations; b) cells with dark contents; c) long, non-glandular unicellular trichomes (Figure 2 D-F).

According to the characteristics observed and comparing with literature data (Varela et al., 2009), the colored irregular fragments were identified as wheat bran. The elongated cells with punctuations corresponded to "cross-cells" of the cereal grain mesocarp. The cells with dark contents were cells containing aleurone and the long trichomes belonged to the exocarp. The greenish color of the fragments was probably due to the addition of a dye.

Table 1
Separate components in oregano commercial samples

"OREGANO" SAMPLES			GENUINE PARTS		FOREIGN MATTER					
Trademark	Net Content (g)	RS (g)	Leaves	Bracts & Flowers	Bran	Other	Total foreign matter			
			%	%	%	%	%	Me	Mean	Var
A	20 20 20	2 2 2	20 30 10	55 60 75	5 0 5	20 10 10	25 10 15	**15**	**17**	**58**
B	25 25 25	2.5 2.5 2.5	12 4 8	14 18 4	68 72 84	6 6 4	74 78 88	**78**	**80**	**52**
C	25 25 25	2.5 2.5 2.5	12 12 12	16 24 32	68 56 48	4 8 8	72 64 56	**64**	**64**	**64**
D	25 25 25	2.5 2.5 2.5	28 32 32	16 60 48	48 0 12	8 8 8	56 8 20	**20**	**28**	**624**
E	25 25 25	2.5 2.5 2.5	68 44 48	8 28 28	0 0 0	24 28 24	24 28 24	**24**	**25**	**5**
F	20 20 20	2 2 2	40 65 45	55 30 45	0 0 0	5 5 10	5 5 10	**5**	**7**	**8**
G	25 25 25	2.5 2.5 2.5	32 40 28	56 48 60	0 0 0	12 12 12	12 12 12	**12**	**12**	**0**
H	25 25 25	2.5 2.5 2.5	24 24 32	70 68 60	0 0 0	6 8 8	6 8 8	**8**	**7**	**1**
I	25 25 25	2.5 2.5 2.5	36 40 32	54 52 56	0 0 0	10 8 12	10 8 12	**10**	**10**	**4**
J	30 30 30	3 3 3	30 28 30	60 60 57	0 0 0	10 12 13	10 12 13	**12**	**12**	**2**
K	15 15 15	1.5 1.5 1.5	40 53 66	47 40 27	0 0 0	13 7 7	13 7 7	**7**	**9**	**12**
L	25 25 25	2.5 2.5 2.5	36 20 24	56 72 64	0 0 0	8 8 12	8 8 12	**8**	**9**	**5**

RS: representative sample; g: grams; %: percentage in relation to representative sample; Other: own and foreign stems; foreign flowers, fruits, seeds; dust, stones, soil particles; Me: median value; Var: variance

Figure 1
Macroscopy of genuine components and foreign matter in commercial oregano

Figure 1
A. Genuine elements: leaves (a), bracts (b), flowers (c), stem fragments (d); B. Foreign matter: bran (arrow) and stems; C. Foreign matter: seeds (arrow) and stems; D. Foreign matter: fruits (arrows), leaves (e) and stems. Scale bar A-D: 1 cm.

Figure 2
Microscopic elements in macerated oregano commercial samples.

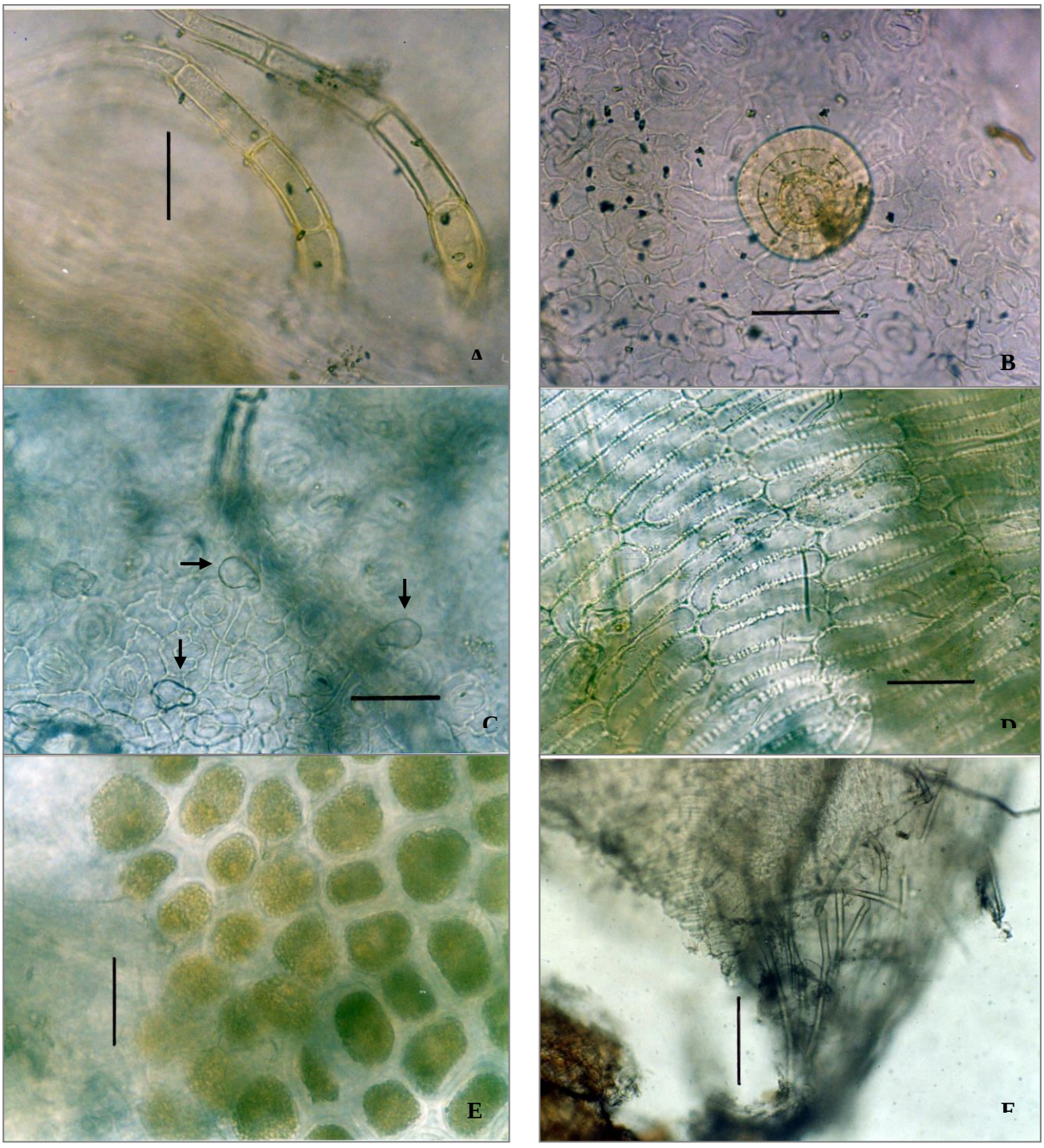


Figure 2
A-C. Genuine elements of "oregano". A: non-glandular pluricellular trichomes; B: glandular peltate trichome; C: glandular capitate trichomes (arrows). Scale bar A-C: 50 µm.
D-F: Bran cereal elements. D: cross- cells of mesocarp; E: cells containing aleurone; F: uni-cellular trichomes of exocarp. Scale bar D-E: 50µm; F: 200 µm.

The genuine leaves of oregano presented different cell types and easily recognizable. The data in the present work coincided with those observed in previous studies (Varela *et al*., 2007), and were also in agreement with those observed in other species of the genus (Rouquaud and Videla, 2001).

Statistical treatment of data

Contents of foreign matter values in the samples were recorded in a spreadsheet of Microsoft Office Excel 2007. The contribution of each trademark to the total foreign matter was represented in Figure 3. As shown, two trademarks (B and C) contributed with more than 50 % of foreign matter, especially bran. The proportions of the plant parts used and the foreign matter in the samples were represented in Figure 4. It is also noted that in trademarks B and C, with high contents of bran, occurred a marked decrease in the plant parts used. The pie and bar charts were performed selecting the median value of the content of foreign matter, which is not influenced by the extreme data present in some samples, resulting in a more suitable parameter of central tendency than the mean.

Figure 3
Contribution of each commercial product to the total of foreign matter found

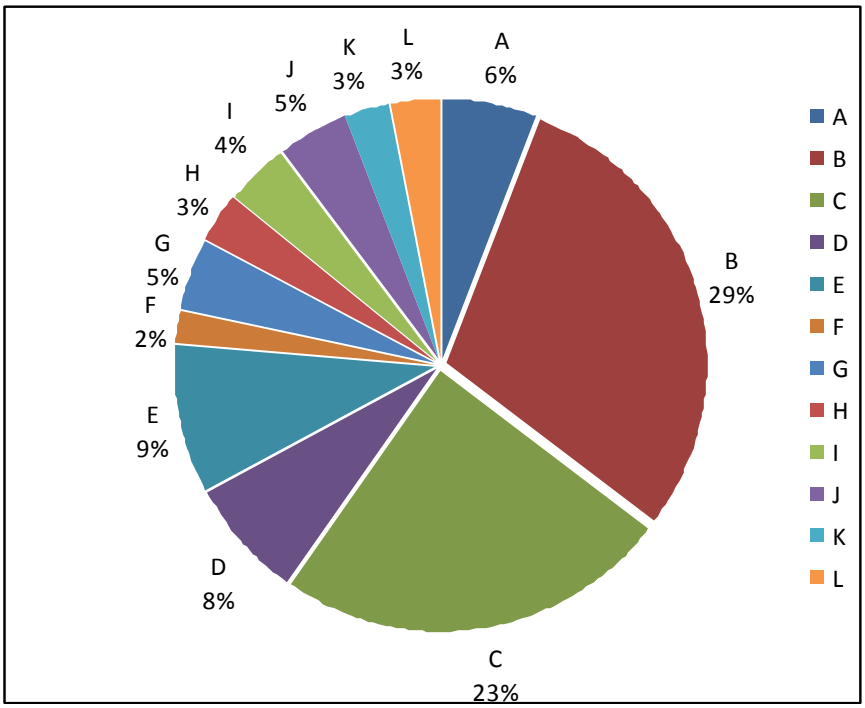
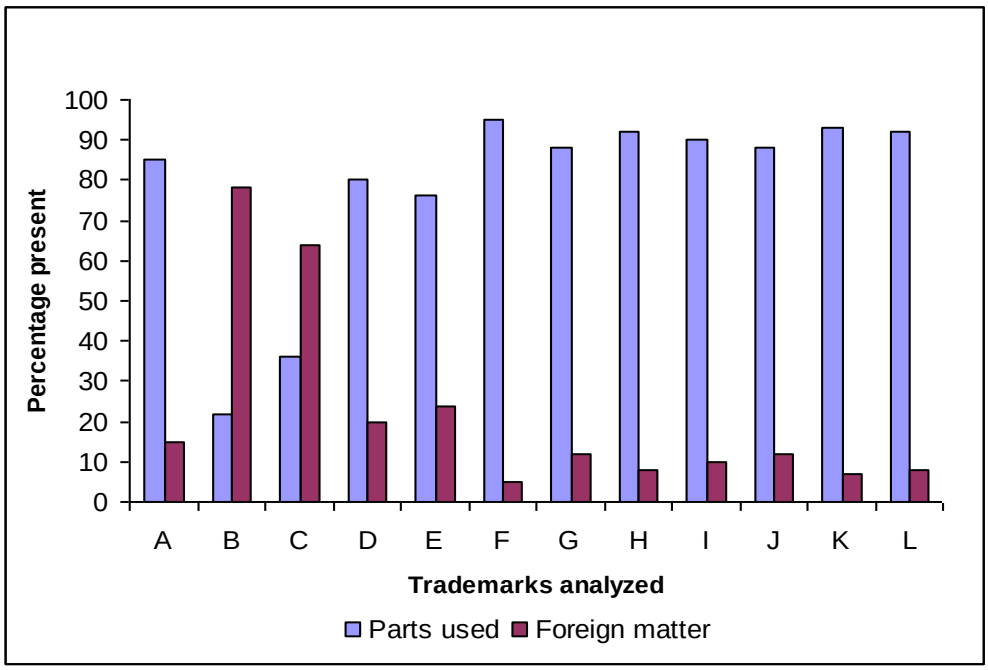


Figure 4
Content of parts used and foreign matter in oregano samples



On the other hand, means and variances of the foreign matter values were calculated and presented in Table 1. A statistical comparison for the different trademarks through a variance analysis using a One-way ANOVA and F-test was performed. The data obtained were summarized in Table 2.

The statistical analyses indicated that the probability (p-value) was much lower than the significance level ($\alpha = 0.05$) and the F calculated value (24.7) was greater than the F critical value (2.2), resulting significant. Therefore, we rejected the null hypothesis that all population means were equal, and assumed that at a 5 % significance level there was strong evidence that existed significant differences in the contents of foreign matter for the trademarks analyzed.

Table 2
Analysis of variance and F-Test with a significance level of 5%. Data from foreign matter values in analyzed samples.

Source of variations	*df*	*MS*	*F cal*	*p-value*	*F crit*
Between groups	11	1721.6	**24.7**	1.98117E-10	**2.2**
Within groups	24	69.8			
Total	35				

df: degrees of freedom; MS: means of squares; F cal: F calculated value; F crit: F critical value.

CONCLUSION

Considering all the above observations, we can conclude that: a) the majority of the samples are not strictly adjusted to the percentages of foreign matter proposed by the CAA (only one of the trademarks satisfies this requirement); b) bran cereal is considered as a substantial adulterant of the samples analyzed; c) some trademarks are not homogeneous in quality,

varying considerably their contents of foreign matter; d) a great decrease in the proportion of the plant parts used is observed in the samples with high contents of foreign elements.

The methodolgy used in this study is quite simple to perform in a medium complexity laboratory, and the elements found contribute to a better control over the oregano for trade.

The proposal is to establish the right methodological training in order to carry out a more exhaustive control of the batches destined to marketing, especially regarding to foreign elements, avoiding the introduction of unexpected adulterants which affects negatively the quality of the commercial product.

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