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Microbiological quality and essential oil of parsley (*Petroselinum crispum*) submitted to the hygienizing and drying process

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ABSTRACT

Parsley (*Petroselinum crispum* Mill.) is a species of wide production in Brazil. In the southern region of the state of Rio de Janeiro, Brazil, it is grown by small farmers with high productivity. Lack of implementation of conservation methods generates economic product losses, for the excess of production. The cleaning has as primary objective to preserve the microbiological quality of food without losing product quality and decreases the health risks to the consumer. Drying is the most used process to ensure the quality of agricultural products. Thus, the objective of this study was to evaluate the cleaning and drying processes of parsley leaves, observing the changes in the microbiological quality and main components of the essential oil. The combined cleaning process with drying at different temperatures provided a greater reduction of the studied microorganisms in relation to separate processes. There was no evidence of the presence of *Salmonella* sp. Drying did not change the oil yield in relation to the fresh plant. The apiole and myristicin were identified as the main compounds of the parsley essential oil.

1. Introduction

The culinary plants are those used as seasoning, enhancing the flavor and aroma, and activating the action of the salivary glands that start the digestive process. Each culinary species has different substances in its composition, which may also have food preservation, medicinal and aromatic properties, such as in the treatment of colds, headaches, respiratory and cardiovascular problems (Cardoso et al., 2005).

Parsley (*Petroselinum crispum* Mill.) is a vegetable species that does not have an important economic value, but it is widely used as a condiment. It is wide-spread throughout Brazil and in the world, possibly being the most universal culinary herb. Its essential oil, obtained from the leaves and seeds, is used as aroma in many fragrances in perfumes. Besides being used in cooking, it has diuretic properties, stimulates menstruation and prevents cardiovascular diseases (Cardoso et al., 2005; Lorenzi and de Abreu Matos, 2002).

Vegetables in general are contaminated with microorganisms on their surface. Therefore, complete cleaning is necessary to make them suitable for feeding, to preserve the purity and microbiological quality of the foods, using good-quality water and with the addition of appropriate sanitizer solutions. One of these solutions can be sodium hypochlorite, a chlorine-chemical-base product, which acts quickly and dissociates completely in water. Thus, it can be widely used in plants in order to maintain microbiological quality (Srebernich, 2007).

Common in the Brazilian market, parsley leaves for flavoring are marketed for both its fresh and dried forms. Drying is the most commonly used commercial process for preserving the quality of food products in which water content, fruit and vegetable activity are decreased by heated air to minimize biochemical, chemical and microbiological deterioration. The major objective in drying food products is the reduction of the moisture content to a level, which allows safe storage over an extended period (Doymaz and Pala, 2003; Martinazzo et al., 2010).

Thus, the aim of the present study was to evaluate the cleaning and drying processes of parsley leaves, to observe changes in the microbiological quality, yield and composition of the main components of the essential oil of the product.

Abbreviations: d.b, dry base; RU, moisture ratio; AOAC, Association of Official Analytical Chemists; APHA, standard plate count agar; GC–MS, gas chromatography–mass spectrometry; WHO, World Health Organization; R^2, coefficient of determination; P, mean relative error; SE, mean error of the estimation; RDC, board resolution

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2. Materials and methods

2.1. Harvest and selection of P. crispum leaves

The plant material used in the experiment was obtained from farmers in the southern State of Rio de Janeiro, Brazil. The leaves were selected, the impurities and extraneous materials removed, and they were subsequently homogenized.

2.2. Chemicals

Chemicals and reagents were obtained from Sigma-Aldrich Brasil Ltda. (São Paulo, SP, Brazil). Pentane, magnesium sulfate anhydrous and hexane standard HPLC were used to extract and analyze the essential oil constituents. Trypticase soy broth, plate count agar and sabouraud agar were used to microbiological analyzes.

2.3. Cleaning process for P. crispum leaves

To evaluate the cleaning process for parsley leaves, two processes were performed, as follows: process A, where the leaves were not subject to any cleaning process, as it is commonly done by farmers, and sold in the Brazilian market; and process B, where the leaves were subjected to washing and sanitizing.

In process B, the samples were first washed in a water stream and then immersed for 15 min in a sanitizing solution of sodium hypochlorite at 200 ppm, and then rinsed twice under a distilled water stream (de Alencar Costa et al., 2012). To carry out the drying process, before being weighed, the excess surface water is removed from the plants through a vegetable manual centrifuge.

2.4. Drying process of P. crispum leaves

To evaluate the drying process, after processes A and B, different temperatures of the drying air (40, 50 and 60 °C) were used, aiming to possibly decrease the necessary time to dry the product, conserving its microbiological quality, and obtaining the best essential oil yield. During the drying process assays, the ambient air conditions of temperature and relative humidity were 26.9 °C and 45.7%, respectively. In addition, the air velocity in the drying chamber was 0.5 m s^{-1}.

A tray dryer equipped with an electric air heating system was used. To obtain the drying curves, periodic weighing was carried out to reach the final moisture content of 0.149 dry base (d.b.).

Before starting the drying process, the water content of the samples was determined by the gravimetric method (ASAE Standards, 2000) for forage and similar plants, by using 25 g of the product, with three replications, in an oven with forced air circulation at 103 ± 2 °C for 24 h.

2.5. Microbiological analyzes of P. crispum leaves

The microbiological quality was determined for micro-organisms, *Salmonella* sp., total coliforms, aerobic mesophilic bacteria, molds, and yeasts on the parsley leaves after undergoing the A and B cleaning and drying processes.

For the research of *Salmonella* sp. and total coliforms, rapid methods were used through a 1–2 Test Kit Simplate, respectively, from BioControl® company (Sovereign Ltda, São Paulo, SP, Brazil), both approved by the Association of Official Analytical Chemists (AOAC). The samples were prepared as described in the manuals.

For aerobic mesophilic bacteria, the standard plate count agar (APHA) culture medium was used, and for yeasts and molds, the Sabouraud Agar medium was chosen. In the samples preparation, 25 g of parsley was weighed and added to 225 ml of sterile pH 7.2 monophosphate buffer. Then, dilutions were prepared to 10^{-5}. The plates containing the APHA medium were incubated at 35 °C for 48 h and those containing the Sabouraud medium were incubated at 30 °C for 48 h.

2.6. Extraction of the essential oil from P. crispum leaves

The extraction of essential oil after processes A and B and drying was made with the hydrodistillation method, using the Clevenger apparatus. Samples of 100 g of fresh parsley leaves were used for the fresh treatment, and 14 g of dried leaves in three replications. The extraction time, counted from the boiling point, was determined through preliminary tests, each lasting 2 h and removed from the hidrolact every 30 min. The results were expressed as the percentage of oil in relation to the dry matter of the product (% d.m.).

2.7. Constituents of the essential oil analysis

Compounds were identified by using the gas chromatography technique coupled with mass spectrometry (GC–MS) in Shimadzu model GC – 2010 equipment (SINC LTDA, Rio de Janeiro, RJ, Brazil) with mass selective detector, model with QP 2010 plus – MS. The chromatographic column used was a fused silica capillary type with DB-5 stationary phase of 0.25 mm thick, 30 m long, and 0.25 mm internal diameter.

The compounds were identified by comparing the mass spectra obtained with the database of the apparatus (NIST and Wiley). The quantitative analysis of each essential oil component, expressed in a percentage, was performed by the normalization method of the integration of peaks area, as described by Zhang et al. (2006).

2.8. Statistical analysis

All the experiments were run in triplicate. The linear regression was performed by Sisvar (Federal Lavras University, Lavras, Minas Gerais, Brazil).

3. Results and discussion

3.1. Microbiological analysis of P. crispum leaves

Table 1 shows the count of microorganisms on fresh and dried leaves in the evaluated temperatures, of the parsley submitted to processes A and B.

For molds, yeasts and mesophilic analysis, there is no established limit in Brazil for their presence in condiments such as parsley. For comparison purposes, the acceptable limits recommended by the World Health Organization (WHO) for medicinal/culinary plants were considered, establishing the maximum of 10^3 and 10^5CFU/g, respectively (WHO, 1998).

For yeasts and molds, in both cases, both fresh and dried parsley showed values above the limit, not being suitable for consumption. For the mesophilic, only the dried parsley in process B from 50 to 60 °C was in accordance with what is suggested by the WHO. High levels of contamination by mesophylls may result in loss of nutritional value and food attractiveness.

Similar results were observed by others authors after evaluating the microbiological quality of medicinal plants such as: Lucca et al. (2010) after evaluating the microbiological quality of chamomile samples (*Chamomilla recutita* L.) coming from pharmacies and supermarkets of the City of Cascavel, State of Paraná, observed mold and yeast values higher than the limit recommended by the WHO and Silva (2002) found mesophilic values higher than 10^5 for parsley and chives, being unfit for consumption

Through the board resolution (RDC) No. 12 of January 2, 2001 (Brasil, 2001), Brazilian legislation specifies that fresh condiments should be free from *Salmonella* sp. This microorganism was not found in any of the processes. Similar results were found, where *Salmonella* contamination was evaluated in vegetables, such as parsley, sold in