

Effect of Disinfection and Drying of Wild Carob Pods (*Neltuma* sp.) on the Safety of the Carob Flour [†]

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Abstract: In the hostile and challenging environment of the Paraguayan Chaco, the wild carob pods (*Neltuma* spp.) are a valuable vegetable resource that provides nutrition and significant economic opportunities for the local populations by means of carob flour production. However, the microbiological quality of the carob flour is limited due to the manual gathering. The main objective of this investigation is to find an efficient disinfectant and its minimum application level to obtain microbial stability in carob flour. The microbial load (total mesophilic aerobes, moulds, and yeasts, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp.) of the flour obtained by disinfection with citric acid (1 and 3%) and sodium hypochlorite (1 and 3%) was compared. Drying tests were carried out at time intervals of 2, 4, 6, and 7 h on whole carob pods to obtain flour in a hot air circulating tray type dryer, and humidity was used as a response variable in a thermobalance (desirable humidity < 5%). A combined process of disinfection with 3% citric acid and hot air circulating tray-type drying for 7 h at 60 °C is proposed to obtain an innocuous carob flour of high microbiological quality.

Keywords: carob; disinfection; drying; moisture; biodiversity; *Neltuma* spp.; *Prosopis* spp.; food safety



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1. Introduction

The fruits of the various species of carob tree in the Paraguayan Chaco are very important for the food and subsistence of different indigenous communities; it occupies a prominent place in the local culture and is appreciated for its significant nutritional value, especially for flour production by milling in artisanal “palo santo” mortars [1]. However, previous studies have shown that quantified levels of yeasts and mesophilic aerobes require attention to ensure the safety of carob pods that are collected from the soil manually before drying for flour production [2], which can be exposed to a variety of harmful bacteria, such as *Salmonella* spp. and pathogenic *Escherichia coli* (E. coli) [3], to fungal contamination *Aspergillus* section *Flavi* and aflatoxins and should be treated to ensure safety prior to commercialisation, as is required for other traditional crops [4].

The main objective of this work was to find an efficient disinfectant and its minimum application level to obtain microbial stability in carob flour.

2. Materials and Methods

2.1. Experiment Development

The samples of carobs collected in the Pykasu community in the Mariscal Estigarribia district, Boquerón Department of the Western Region, Paraguayan Chaco, were used. They were transported under appropriate conditions at room temperature to the Industrial Microbiology Laboratory of the Research Department of the Faculty of Chemical Sciences of the UNA. First, the washing of whole carob pods was studied under six conditions: citric acid 1% and 3%, sodium hypochlorite 0.1% and 0.3%, rinse with water, and without rinsing (control).

Each solution had a contact time of 30 min with the carob pods and a draining time of 30 min. Subsequently, all groups were treated with the same method of drying. A dryer was used in trays with forced air circulation at a constant temperature of 60 °C until reaching 5% humidity in accordance with current regulations for carob flour [5]. The dryer equipment was designed and built in the Department of Industrial Applications of the Faculty of Chemical Sciences, equipped with (a) a helical fan, (b) a table contactor, (c) an internal resistance type heater, and (d) a drying chamber with perforated plates, which consists of fans for the supply of fresh air and an electric resistance heater. The surface air velocity was measured with an anemometer (HoldPeak® model HP 866B, wind speed accuracy $\pm 5\%$, Zhuhai, China), digital thermometer (Extech Instruments, model 421305, Waltham, Massachusetts, USA), air humidity is controlled with a digital thermohygrometer (BOE 330), and drying time with a digital stopwatch (Huawei Band 6-7E5). During all hot air-drying experiments, the airflow direction was parallel to the samples. The microbiological analysis was performed on all flours obtained by milling the dry pods and then sieving them to a particle size of 100 μm .

2.2. Analysis

Moisture, total solids in flour, mesophilic aerobic count, mould and yeast count, *E. coli* counts, and *S. aureus* were determined by AOAC official methods [6]. *Salmonella* spp. was determined by ISO 6579-1:2017 [7] by counting on a plate. Microbiological parameters such as mesophilic aerobic count, yeast and mould, count *Escherichia coli* count, *Staphylococcus aureus* count, and the absence of *Salmonella* spp. were confirmed; each of the determinations was made in triplicate. The result of the counts was expressed in CFU (colony-forming units)/g. In the case of *Salmonella* spp., the result was expressed as absence/25 g.

3. Results and Discussion

Method of Disinfection and Microbiological Counting of Carob Flours

In Table 1, the microbiological results of the samples of carob pod flour subjected to rinsing with different disinfectants obtained by drying in trays with forced circulation air at 60 °C for 7 h are presented. According to the Peruvian Technical Standard NTP 209.602 Carob Flour: Definitions and Requirements [5], the maximum value of mesophilic aerobic count, mould and yeast count, *E. coli* counts, and *S. aureus* counts is 1×10^2 CFU/g, and for *Salmonella*, absence is required. The counts of mesophilic aerobic count applying disinfection of 3% citric acid and 0.3% hypochlorite are within the parameter established by the standard used, but not for the parameter of counting mould and yeast count and *Staphylococcus aureus* count, which applying the disinfection of 0.3% hypochlorite is not within the parameter established by the standard. The microbiological parameters for carob flours for mesophilic aerobic count, mould and yeast counts, *E. coli* count, *S. aureus* count, and *Salmonella* spp. [5] were in the acceptable range with treatment in a 3% citric acid solution.

Table 2 shows the results obtained for the determination of moisture in the flours obtained in all treatments. Drying maintained for 7 h achieved a humidity of less than 5% in all the samples analysed, with no significant differences in the means (ANOVA, Tuckey post-test $p < 0.05$).

This research proposes an effective method of disinfection to obtain carob flour (*Neltuma* sp.) with microbiological quality under local conditions, which must be adjusted for different species of carob trees that present morphological differences that may influence the drying process. This tradition of taking advantage of the various fruits from the Chaco mountain has deep roots in ancestral history. The carob flour can be an excellent alternative for gluten-free muffins and baked goods, where, in general, the technological quality is not affected in the resulting products (except for a smaller volume in the dough), but they improve in the extension of shelf life by reducing the retrogradation of starch as a result of the modification of the gluten protein matrix exerted by the dietary fibre and globular proteins of carob flour [8]. Thus, the carob fruits of the Paraguayan Chaco have a great

potential for use at a nutritional and industrial level within the framework of Food and Nutritional Security and are necessary conservation programs and management plans for the sustainability of this food resource.

Table 1. Results of the determination of mesophilic aerobes, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., moulds, and yeasts in samples of carob pod meal.

Variables	Mesophilic Aerobes (CFU/g)	Moulds and Yeasts (CFU/g)	<i>E. coli</i> (CFU/g)	<i>Staphylococcus aureus</i> (CFU/g)	<i>Salmonella</i> spp. (CFU/g)
Citric Acid 3%	2×10	<10	<10	1×10	Absence/25 g
Citric Acid 1%	1×10^4	<10	<10	1×10^2	Absence/25 g
Sodium hypochlorite 0.3%	5×10	2.5×10^2	<10	1×10^3	Absence/25 g
Sodium hypochlorite 0.1%	1×10^3	3×10^2	<10	2×10^2	Absence/25 g
Rinse with Water	4×10^3	1×10^4	<10	3×10^3	Absence/25 g
No rinse/Undried	1×10^3	1×10^6	<10	1×10^4	Absence/25 g
Max. value Reference (CFU/g)	1×10^2	1×10^2	1×10^2	1×10^2	Absence/25 g

Reference: Peruvian Technical Standard NTP [5]. CFU: Colony-Forming Units.

Table 2. Moisture at different drying times by trays with forced air circulation.

Time	Moisture (%)
2 h	7.03 ± 0.02
4 h	6.32 ± 0.03
6 h	5.48 ± 0.02
7 h	4.80 ± 0.03

The results are expressed as an average and their corresponding standard deviations ($X \pm DS$).

4. Conclusions

Washing the whole pods with 3% citric acid was optimal for the count of microorganisms analysed: Mesophilic aerobes, fungi, and yeasts; *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp. It was observed that the combined process of disinfection and drying in trays with forced air circulation for 7 h at 60 °C has a positive effect on the microbiological quality of the flours obtained and complies with the humidity standards necessary for their marketing and consumption. This reinforces the position of carob as a viable raw material for the production of safe food.

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