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Microbiological assessment of gourd for chimarrão: strategy for health prevention

Avaliação microbiológica de cuias para chimarrão: estratégia para prevenção da saúde

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ABSTRACT

Introduction: The gourd, the preparation vessel of *chimarrão*, can be a propitious medium for the proliferation of microorganisms. **Objective:** To analyze the presence of microbiological contamination in the gourds used for *chimarrão*, and the effectiveness of the use of a drier in the gourds. **Method:** Twelve used and twelve new gourds were used; they were divided into drying groups, one with drier and another one without drier. All remained 14 hours per day with the infusion. Three mL of Brain Heart Infusion (BHI) were added to each gourd, collected, incubated for 24 h at 37°C and then 1 mL was seeded in triplicate in Sabourand and Plate Count Agar (PCA) media. This analytical methodology was used on days 0, 15 and 30. **Results:** Fungal growth, evaluated by the Sabourand medium, was statistically different between the new gourds on Day 01 (p = 0.004); on PCA there was difference on Day 01 (p = 0.004) and Day 15 (p = 0.046) between new gourds and on Day 30 (p = 0.004) for the used gourds. **Conclusions:** Contamination occurred in the material analyzed, which could represent a risk to the health of its consumers; the use of the drier was not effective to avoid the growth of microorganisms.

KEYWORDS: Gourd; Microbiology; Assessment; Public Health

RESUMO

Introdução: A cuia utilizada para consumo do chimarrão pode ser um meio propício para a proliferação de microrganismos, o que pode comprometer a saúde de seus consumidores. **Objetivo:** Analisar a presença de contaminação microbiológica nas cuias utilizadas para o chimarrão, e a eficácia do uso de um secador nas cuias. **Método:** Foram utilizadas 12 cuias usadas e 12 novas, as quais foram divididas em grupos de secagem com secador e ao natural. Todas permaneceram 14 h por dia com a infusão. Foram adicionados 3 mL de *Brain Heart Infusion* (BHI) em cada cuia, coletados, incubados por 24 h a 37°C e, então, 1 mL semeado em triplicata nos meios Sabourand e *Plate Count Agar* (PCA). Esta metodologia analítica foi usada nos dias: 0, 15 e 30. **Resultados:** O crescimento fúngico, avaliado pelo meio de Sabourand, foi estatisticamente diferente entre as cuias novas no Dia 01 (p = 0,004), no PCA houve diferença no Dia 01 (p = 0,004) e Dia 15 (p = 0,046) entre as cuias novas e no Dia 30 (p = 0,004) para as usadas. **Conclusões:** Ocorreram contaminações no material analisado, o que pode representar um risco para a saúde de seus consumidores, e o uso do secador não foi efetivo para evitar o crescimento de fungos e leveduras.

PALAVRAS-CHAVE: Cuia; Microbiologia; Avaliação; Saúde Pública

INTRODUCTION

Food safety is important because of its relation with public health. It has been linked to the physicochemical and microbiological quality of products, which must remain intact and without contamination throughout the production chain until they reach the end consumer[1,2,3].

Products or materials with water are susceptible to the growth of microorganisms (fungi, yeast and bacteria)[4,5]. To prevent that, microbiological control can be done through physical and chemical methods. However, when choosing one of the methods, some important aspects must be considered, like: whether the method is efficient, whether it damages or interferes with the material to be sterilized, the convenience of the method, its cost and damage to the environment or the materials[6,7].

In order to minimize the aforementioned risks of contamination, chemical disinfectants like chlorine are widely used in the food industry to reduce undesirable microorganisms. Another well-known method is sterilization by ultraviolet (UV) light, but the use of visible light has also shown bactericidal effect under certain conditions[8,9,10]. These methods are specific for certain products or materials. When it comes to gourds, scientists are looking for new methods to improve food safety, especially microbiological control[3].

Mate tea, also known as *chimarrão*, is a traditional beverage in southern Brazil. It was originally consumed by native populations of Argentina, Paraguay and from the Brazilian states of Paraná and Rio Grande do Sul. It is prepared with a medicinal plant called *Ilex paraguariensis*, or *yerba mate*, as an infusion in water. For its consumption, a specific container is used: the *mate* gourd bowl or simply gourd (*cuia*, in Portuguese)[11,12]. According to Barté et al.[13], 90% of this plant is used to make *mate* and the states of Paraná, Santa Catarina and Rio Grande do Sul are the largest producers of this crop.

The gourd is made from *porongos*, the inedible fruit of *Lagenaria siceraria*. The container is made of the fruit's thick, pulp-free shell. Its use, although traditional, has been questioned due to possible microbial contamination, since it is an organic material in constant contact with aqueous medium and there is no quality control of microorganisms in these containers required by the legislation. *Mate* is consumed daily by a large portion of Rio Grande do Sul's population, so this topic is relevant and necessary[12,14,15].

Considering the above and the fact that there are no studies on microbial growth in gourds, the aim of this research was to evaluate the microbiological contamination of new and used gourds dried by a device designed for this purpose and by the traditional method.

METHODS

We used 24 gourds. Twelve were new and twelve had already been used. The new gourds had been polished, treated with nitric acid, heated, waxed, and industrial talcum was applied to their surface. According to the methodology described by Pinheiro, Wanda and Pereira[16], we established that the gourds used for at least 90 days were considered used. We performed the experiment between June 2 and June 29, 2017. We recorded temperature and moisture values every day, with a thermo-hygrometer certified by the Brazilian Institute of Metrology, Quality and Technology (Inmetro). The procedures were always performed at a standardized time, and all the aforementioned variables were measured.

The gourds were prepared daily according the following steps:

1. Punctually at 5 pm, from Monday to Saturday, the *mate* was prepared in every gourd, with 20 g of fresh *yerba mate* (*I. paraguariensis*), without contact with air, heat or moisture, and 100 ml of deionized water at room temperature.

2. After preparation, we kept the samples in a fume hood so that it did not come into contact with any interferers.

3. We cleaned the gourd at 7:30 am on the following day, thus leaving it infused for 14 hours. Next, we dried it until 9 am.

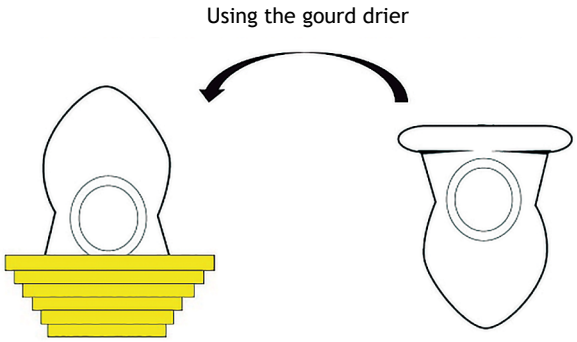
We cleaned all the gourds with tap water and removed any remnants of *yerba mate* or water from the previous day. We dried the gourds in different ways, depending on the gourd. In 12 gourds we used the drier designed for this research project. Six of them were used and six were new. The other 12 gourds (also six used and six new) dried normally in the environment with their top facing up. The drying time for the gourds was 2 hours. Then, we put them away until the next procedure on the same day.

The drier used was made of plastic and had a 10 Watt incandescent light bulb in its center. It also had several levels in which we put the gourds with different diameters, as shown in Figure 1.

For the microbiological analysis, we added 3 mL of Brain Heart Infusion (BHI) culture medium in all gourds with pipettes. This medium is used to cultivate fastidious bacteria, filamentous fungi and yeast. We put the BHI medium for one minute in the gourd inner surfaces, collected 1 mL of it and stored it in properly identified sterile test tubes, as shown in Figure 2.

Afterward, we incubated these tubes for 24 hours at 37° C. After incubation, we plated 10 µL of this culture on Petri dishes with Sabourand, MacConkey and Plate Count Agar (PCA) media. Sabourand culture medium allows mold and yeast growth, MacConkey, enterobacteria, and PCA, mesophilic microorganisms[17,18]. We plated and counted the microorganisms at three different times: on the 1st day (Day 1), 15th day (Day 15) and 30th and last day (Day 30).

In the first collection period (time 0), we plated 72 Petri dishes, 24 of which with Sabourand, 24 with MacConkey and 24 with PCA media. Then, we put them in the incubator for 24 hours at 37° C[19]. To standardize the reading of the plates, we established



Source: From the author.

Figure 1. Gourd drier and its use process.

the following denominations: Negative (N), there was no macroscopic growth of colonies; Countable (C), there was macroscopic growth of up to 130 colonies; Countless (I), there was macroscopic growth of more than 130 colonies[20].

The only change we made for the other analysis periods was the exclusion of the MacConkey medium, since it did not show significant growth and, therefore, little contamination.

For data analysis, we used descriptive statistics procedures like absolute and relative frequency. We tested the normality of the data using the *Kolmogorov-Smirnov* test. To verify the association between two or more qualitative variables, we used the Pearson's chi-squared hypothesis and Fisher's exact test. For all tests, we considered a significance level of 5%. We used the version 18 of the Statistical Package for the Social Sciences (SPSS) software to analyze the data. For analysis purposes, we considered presence or absence of growth. We did not use number of colony forming units.

RESULTS

Among the three microbiological evaluations we performed, we found microbial growth in MacConkey medium – therefore, enterobacteria – in only one used gourd on the first day of analysis. In Sabourand medium, the new gourds that used the drier showed higher colony growth compared to those that had not been in the drier. The same was true for the used gourds in this culture medium. These data are shown in the Table.

Nevertheless, in PCA medium, the new gourds that did not use the drier showed higher colony growth compared to those that did. The used gourds that used the drier presented the highest growth. Fungal growth, evaluated by Sabourand medium, was statistically different among the new gourds only on Day 1 ($p = 0.004$). With PCA, there was a difference between Days 1 ($p = 0.004$) and 15 ($p = 0.046$) for the new gourds, and on Day 30 ($p = 0.004$) for the used ones.

It is noteworthy that on the first day of the experiment the gourds had not been dried yet and we used the results of this analysis to compare it with the data of the 15th and 30th day, presented in the Table. Among the Sabourand Petri dishes, we found that drying minimized fungal contamination of the used gourds on Day 30, with significant difference. In PCA medium, we also found this difference in the used gourds on Day 30 ($p = 0.04$). We found that using the drier to prevent the growth of fungi and yeast was not effective, since there were no significant results if we consider the comparison between new and used gourds, with and without the drier. During the experiment the average temperature and environment humidity rate were 16.5° C and 68.7%, respectively.

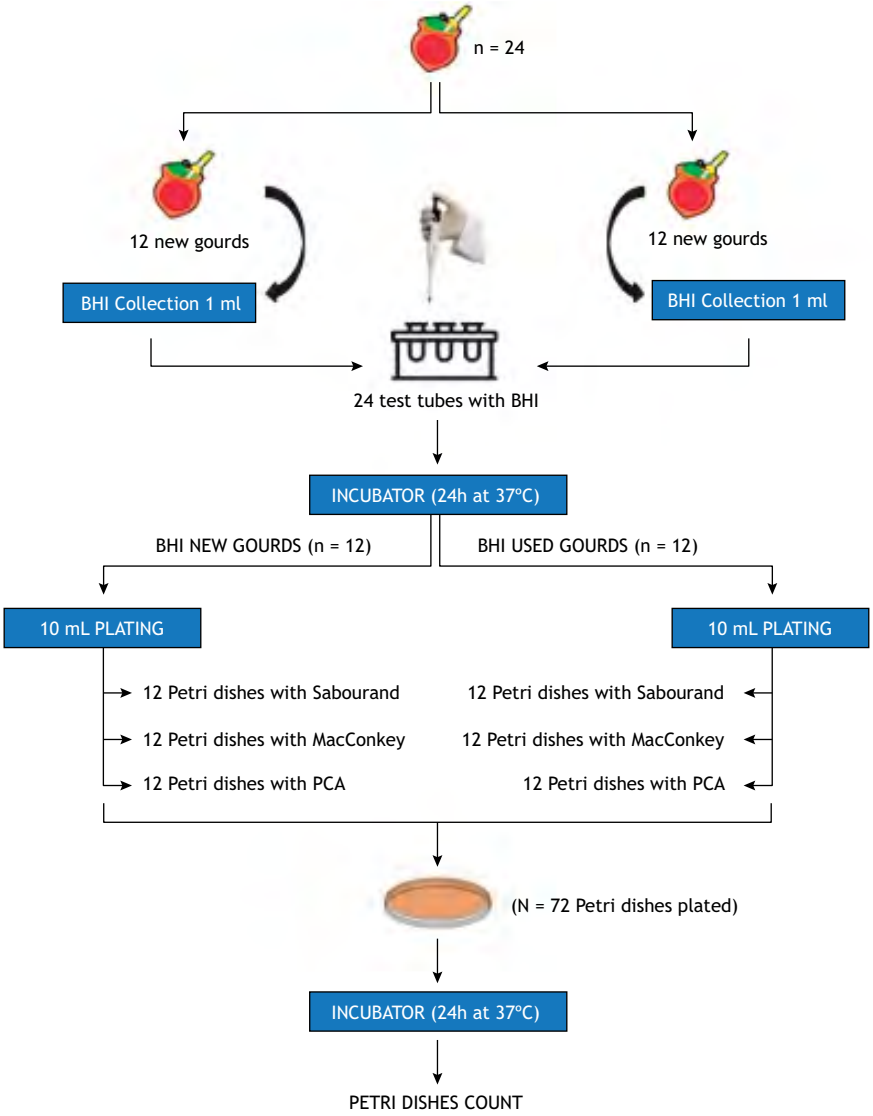
DISCUSSION

There are several factors that increase microbial growth when we are handling *mate*. The gourd has a porous surface and therefore it absorbs liquids that may remain there for a long time, along with the organic material (*yerba mate*) infused with hot water. In addition to that, the beverage can be shared with other people and this may increase bacterial transmission and contamination[4,11,16]. Factors that increase contamination and proliferation of microorganisms on surfaces are the bacteria's adherence to the surface of the material, its chemical composition, surface load, rigidity or physical configuration, temperature and oxygen[21,22,23].

Fungi and bacteria are widely distributed in nature, especially in decaying organic substances[4,18], including *yerba mate*. In the present study, using the Sabourand medium, there was a greater growth in the new gourds, showing that, as they are made of more recent organic material, they retain more moisture and present a more favorable environment for microbial growth. However, when comparing the results of the new gourds that used the drier to those that did not, we noticed minimal growth difference. These results demonstrate that drying was not an effective method to prevent the growth of fungi and yeast in the gourds.

The proliferation of the microorganisms present in a food depends on some characteristics of the food itself (intrinsic factors), like acidity (pH), water characteristics and interactions between the microorganisms. In addition, there are factors related to the environment in which the food is found (extrinsic factors), like moisture, weather, temperature and the chemical composition of the atmosphere surrounding the food[7,24].

The optimal yeast growth temperature is usually between 20° C and 30° C. Molds are, like yeasts, a very heterogeneous group of fungi. Controlling air moisture is very important to prevent mold proliferation. Most molds grow well at temperatures between 15° C and 30° C and the optimal temperature is between 20° C and 25° C[4,6]. In our study, the average temperature during the experiments was 16.5° C, that is, lower than what is considered adequate for microbial proliferation. However, air moisture reached about 68.7% and the ideal percentage is around 70% to 100%[25,26]. This factor may have contributed to the growth of molds and yeasts on most of the new gourds that used the drier.



BHI: Brain Heart Infusion; PCA: Plate Count Agar.

Figure 2. Flowchart of the methodology used to evaluate gourds in growth media.

Table. Analysis of microbial growth in new and used gourds using the drier or the traditional method.

Culture media/drying method		Type of gourd/Period					
		New			Used		
		Day 1	Day 15	Day 30	Day 1	Day 15	Day 30
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Sabourand							
With the drier	Negative	1 (16.7)	3 (50.0)	1 (16.7)	3 (50.0)	6 (100.0)	0 (0.0)
	Positive	5 (83.3)	3 (50.0)	5 (83.3)	3 (50.0)	0 (0.0)	6 (100.0)
Traditional	Negative	0 (0.0)	6 (100.0)	0 (0.0)	4 (66.7)	6 (100.0)	0 (0.0)
	Positive	6 (100.0)	0 (0.0)	6 (100.0)	2 (33.3)	0 (0.0)	6 (100.0)
p		0.004*	0.175	0.004*	0.611	-	-
PCA							
With the drier	Negative	1 (16.7)	3 (50.0)	0 (0.0)	2 (33.3)	0 (0.0)	1 (16.7)
	Positive	5 (83.3)	3 (50.0)	6 (100.0)	4 (66.7)	6 (100.0)	5 (83.3)
Traditional	Negative	0 (0.0)	0 (0.0)	0 (0.0)	4 (66.7)	0 (0.0)	0 (0.0)
	Positive	6 (100.0)	6 (100.0)	6 (100.0)	2 (33.3)	6 (100.0)	6 (100.0)
p		0.004*	0.046*	-	0.540	-	0.004*

PCA: Plate Count Agar; Negative: gourds in which there was no microbial growth; Positive: gourds in which there was microbial growth (countable or countless).
*p < 0.05: significant difference between the results; p> 0.05: no significant difference between the results.

Exposure to high temperatures is one of the most widely used techniques to ensure microbiological stability or even commercial sterility of food. Drying is also one of the oldest processes used by humans in food preservation[3,5,7,26]. The preservation of food by drying is based on the fact that microorganisms and enzymes need water to function. The drying performed by applying heat destroys microorganisms by coagulating their proteins and enzymatic systems[27]. Based on this premise, we evaluated the techniques of drying and sterilization of gourds. However, the results show that the drying method we adopted is not effective, probably due to the fact that it used a temperature below 100º C. To ensure sterilization of food stored at room temperature (usually below 40° C) of pathogenic bacteria or food spoiling microorganisms, it is necessary to apply temperatures between 110° C to 120° C[26], which were not reached by the gourd drier we designed.

According to Eleftheriadou, Pyrgiotakis and Demokritou[3], various methods are available on the market to reduce microorganisms in food and food products. These methods include heat, freezing, radiation (UV, gamma), filtration, drying, chlorine, ozone and hydrogen peroxide compounds. In parallel with our study, Bagci and Temiz[8] determined the sanitizing effects of hot water, chlorine and hydrogen peroxide on the surfaces of oranges inoculated and non-inoculated with *Escherichia coli* ATCC 25922. The authors reported that hydrogen peroxide and water were effective to reduce strains of *E. coli*, and that a short immersion in hot water without chemicals is an effective method to reduce the microbial population on surfaces.

The combined use of UV radiation and chemicals shows a synergistic effect on the control of microorganisms[10]. LED light has also been used for food decontamination through inactivation of a variety of significant foodborne pathogens with minimal heating effect. Therefore, LED can be used with cold storage methods[9] and may be an alternative to be tested in lieu of the standard bulbs used in the present study.

No other studies addressing the contamination of gourds were found in the databases other than a summary presented at the XXXI Integrated Academic Journey of the Federal University of Santa Maria, state of Rio Grande do Sul, Brazil. Lohmann[28] evaluated the microbiological contamination of the gourd that came from a family home and found that the sample generated carpet shaped microbial growth and more than 300 colony forming units (CFU). The author also reported that the gourd may have been first contaminated during the manufacturing and/or commercialization processes, since there are not standardized treatments for that.

Koroglu et al.[29] compared keyboards and touchscreen mobile phones/devices as a potential risk of microbial contamination. 205 mobile phones were tested for microbial contamination. 143 (97.9%) of the touchscreen and 58 (98.3%) of keyboard devices showed microbial contamination. That study demonstrates how important surface cleaning processes are to prevent microbial growth. Compared to the present study, the risk of gourd contamination is higher, since *mate* is considered to be food.

A microbiological study of surfaces in a restaurant in the city of Palmas, state of Tocantins, Brazil, identified bacterial species that can cause gastrointestinal disorders[30]. Another study done at a college in the city of São Paulo, state of São Paulo, Brazil, analyzed food cutting boards and found aerobic mesophilic organisms, molds, yeasts and enterobacteria in 90% of the samples[16]. As with such materials, we found colony growth in at least one gourd of each group, except for the 15th day using Sabourand medium. These results demonstrate that the gourd is a suitable environment for the proliferation of pathological agents.

There are gourds made of different materials available on the market, however, the most common are those made from *porongos*. Considering that the daily consumption of *mate* is a very common habit in most of southern Brazil, northern Argentina, Paraguay and Uruguay[11,12,14,15], doing it in a safe and healthy manner, without risk of microbiological growth, must be a priority of the health system and, for that, strategies must be considered, including driers or associated methods.

Another factor that may contribute to the contamination of gourds is the quality of the water used in the *mate*, since the water can favor the proliferation of microorganisms. Data from the World Health Organization (WHO) show that 80% of the diseases in developing countries are caused by contaminated water[31]. The water used in the present study underwent quality tests, therefore, this was not an interference factor in the analysis we performed.

The results of this study demonstrate the need to publicize the importance of gourd hygiene and to think of strategies to improve gourd asepsis and prevent gourds from becoming bacterial culture media that may harm the health of their users. With respect to drying, suitable practices must be used, like methods that quickly remove excess moisture from the gourds, because, as shown in the Table, moisture can be susceptible to the growth of bacteria and yeast. This is an important topic to be addressed in further studies.

CONCLUSIONS

The use of the drier for better gourd sterilization had no positive effects because there were insignificant colony growth differences between the gourds that were dried and those that were not. New gourds have shown higher microbial growth compared to used gourds, in all culture media we used. Sabourand and PCA media had a slight decrease with the use of the drier, but that did not extinguish microbial growth.

Therefore, better, more effective and feasible methods for drying and sterilizing gourds, aiming to reduce the contamination by bacteria and fungi and, consequently, the diseases they cause, must be developed. *Mate* is a good medium for microbial growth, so hygiene care and control of contaminating factors are key for maintaining the tradition with health and quality of life.

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Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.