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# Investigating the Microbial Indicators of Extracted Chubak Juice for Halva Tahini Preparation: Insight into Production Methods

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***ABSTRACT***

**Background**: Extracted Chubak juice (*Ab-chubak*) is used for aeration, whitening, and increasing the consistency of Halva Tahini. This study investigated the most important microbiological quality parameters of *Ab-chubak*. **Methods:** Sampling of *Ab-chubak* was done from 100 Halva Tahini units (manual and boiling preparation methods) in Ardakan city. The serial dilution and pour plate method on Sabouraud Dextrose Agar were used for fungal detection, as this medium is primarily employed for the selective cultivation of *yeasts* and *molds*, and additionally for determining the microbial contamination in food. Total coliforms (TC) and Fecal coliforms (FC) were identified by most probable number (MPN) method. **Results:** *Yeast* frequencies were 31.6% and 48.8% in the manual and boiling of *Ab-chubak* production methods. Similarly, the results of *Geotrichum* and *Aspergillus* fungi were 31.6% and 10% in manual methods, respectively, whereas in the secondary method, *Geotrichum* had frequency of 11.6%, and *Aspergillus* was not detected. TC were 392.52 and 508.25 MPN/100 ml in *Ab-chubak* prepared in manual and boiling methods, while FC coliforms were 308.31 and 434.55 MPN/100 ml in the mentioned samples, respectively. The results indicated a significant difference in mean number of fungal colonies between two Ab-chubak preparation methods ($P$=0.04). Also, the results showed a significant difference ($P$=0.002) in the morphology of the investigated fungi in two *Ab-chubak* preparation methods. **Conclusion:** The average of bacterial indicators was similar in two *Ab-chubak* preparation methods. However, the presence of fungal species such as *Aspergillus* was confirmed, which can be considered as an opportunistic pathogen.

## Introduction

Recent data from the CDC shows that around 48 million Americans experience foodborne illnesses annually, leading to 128,000 hospitalizations and 3,000 deaths (Mahasti

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Shotorbani and Momeninejad, 2019). This is a major issue in public health that can be mostly avoided. There is a strong possibility of food being contaminated with different types of fungi, resulting in the development of mycotoxins during food production, preparation and storing processes (Akbarian and Babaei, 2019). While developing food, fungi decrease both the quantity and nutritional value of food by removing contaminated portions and affecting its nutrients. These microorganisms produce mycotoxins, secondary metabolites with significant impacts like causing cancer, birth defects, stunting growth, weakening the immune system, and inducing genetic mutations in organisms (Henao *et al.*, 2010).

Coliform bacteria and *Escherichia coli* are the other important indicators of food safety and hygiene. Their presence in food and beverages often signifies poor sanitation practices (Sholichah *et al.*, 2022). Non-*Escherichia coli* may also pose potential health hazards, as they can colonize the human intestine and produce enterotoxins (Twedt and Boutin, 1979).

Monitoring coliform levels can help validate compliance with food safety regulations and assess the potential presence of related pathogens. However, in some processed food items, more resistant organisms may serve as supplementary markers. Overall, controlling coliform contamination is essential for maintaining food quality and preventing foodborne illnesses (Mossel and Struijk, 1995).

Halva Tahini is a well-known product in Iran, Turkey, Lebanon, Greece, and Saudi Arabia. In Iran, soapwort extract (from Saponaria officinalis) is used to enhance its texture and color, while in other countries, they tend to rely on ingredients like glucose or egg whites instead (Shaker Ardakani *et al.*, 2012). Due to its nutritional content, ease of consumption, and affordable price, it is especially popular in hot and dry regions as a high-energy food source. In the production steps, proportions of sugar-glucose mixture, *Ab-chubak*, and Tahini are combined in such a way that a solid content is formed from the emulsion of sesame oil (Tahini) in the sugar syrup mixture (Alpaslan and Hayta, 2002, Arslan *et al.*, 2005).

Chubak is the popular term for certain plants belonging to Caryophyllaceae family. The Chubak plant is characterized by its warm and dry nature, and it is a compact shrub. Acanthophyllum genus plants, characterized by being shrubby with thick roots, are predominantly located in Iran (Hossini tabatabaei *et al.*, 2017). The Saponaria genus includes other plants that are herbaceous and can be annual or perennial, most commonly found in Europe and are sometimes seen in Iran. Saponins and saponin extracts from plants are utilized for multiple purposes in food industry. In Iran and Turkey, Chubak extract and other similar plants are used as emulsifiers in the preparation of halva (Sezgin and Artik, 2010). Several research studies were conducted with the aim of structure identification and physicochemical and biological properties determination (Azadbakht *et al.*, 2005, Gaidi *et al.*, 2004). Saponins and saponin extracts of plant origin are used for various purposes, especially in food industry (Güçlü-Üstündağ and Mazza, 2007). For example, to achieve a whiter color, prevent oil separation from the mixture, improve textural properties, and increase the volume of Halva Tahini, an extracted liquid of saponin (*Ab-chubak*) is added to the contents.

*Ab-chuback* is produced in two ways: Manual and boiling methods. In the manual process, Chubak plant is mixed with water and preserved in plastic containers. After several months, the produced extract (*Ab-chubak*) is ready for use. in contrasted the boiling method, involves the extract is produced via heating Chubak plant. Since this extract is stored in a refrigerator, it has a longer shelf life and better preservation capabilities.

As standard NO. 2695 guidance, Escherichia coli should be negative in 1 g of Tahini sample, consequently the coliform count should not exceed 50 CFU per 1 g, and the *mold* and *yeast* should not exceed 100 CFU per g of Tahini sample.

So far, studies have been conducted on the microbial parameters of Halva Tahini, but no survey has been investigated on *Ab-chubak* used in Halva Tahini preparation. The results of this study can determine the necessity of developing



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standards for this food product. Therefore, the main objective of this research was to determine the most important microbial parameters, with an emphasis on identifying characteristics of fungi present in the extracted choubak juice (Abchoubak).

## Materials and Methods

### *Sample size and characterization*

In this study, the sample size was initially estimated using the following formula (Machin *et al.*, 2011).

$$n_1 = n_2 = \frac{(p_1(1-p_1)+p_2(1-p_2))(Z_{1-\frac{\alpha}{2}}+Z_{1-\beta})^2}{(p_1-p_2)^2} \quad \textbf{Equation 1}$$

*n: Sample size*
*p: Proportion of fungi in preparation method*
$Z_{1-\frac{\alpha}{2}}$ *: Significance level* $\alpha$
$\alpha$*: Critical value (for 95% confidence level)*
$Z_{1-\beta}$*: Test power* $1-\beta$
$\beta$*: Probability of Type II error (for 80% power)*
*Using Equation 1, the sample size of 50 was calculated. So, the number of samples was 50 for each manual and boiling preparation methods, separately.*

Ab-chubak is a brown liquid with relatively high viscosity that is added to Halva Tahini to prevent oil separation from the texture and to improve its characteristics. **Figure 1** shows the used Chuback plant and Ab-chuback in the present study.


**Figure 1.** The Chuback plant (A) and prepared Ab-chuback (B)

### *Microbial test performance*

As microbial national standards, Total coliforms (TC), fecal coliforms (FC) and fungal parameters were selected to examine in the present study.

For fungal test examination, samples were transferred to the laboratory. The *molds* and *yeasts* enumerations were performed by the colony counting method. The primary suspension (initial dilution) and other dilutions were prepared according to the Iranian National Standard No. 10899-3, using the method of serial dilutions and pour plate count in Sabouraud Dextrose Agar (SDA) medium. Then, Plates were incubated at 25 °C for 3 to 5 days (Frances *et al.*, 2021, Iranian National Standardization Organization, 2013).

Colonies were also investigated as *yeast* or *mold* cells by an optical microscope. For the morphological survey, a piece of grown fungus was prepared with lactophenol reagent and investigated under high magnification microscope (Ajobiewe, 2021, Elgarhi *et al.*, 2020, Iranian National Standardization Organization, 2013). The morphology of fungal genera, such as *Geotrichum* and *Penicillium*, was examined with respect to their distinct characteristics: *Geotrichum* colonies typically appear creamy, yeast-like, and arthrosporic, often with a powdery surface, while *Penicillium* exhibits characteristic brush-like conidiophores and powdery green or blue-green colonies. Mentioned characteristics were confirmed microscopically, aligning with standard mycological identification protocols (Samson, 2011)

For bacterial test examination, TC and FC examinations were conducted as No. 2946 and 11166 of national standards (Institute of Standards and Industrial Research of Iran, 2008, 2021). The multiple-tube fermentation method was used for coliform detection. Initially, 0.1 ml of the sample was added to tubes containing 10 ml of Lauryl Sulfate Broth (LSB broth) at standard concentration. The inoculated media were incubated at 37 °C for up to 48 hours, and the gas production was checked at 24 and 48 hours. In the presence of turbidity and gas in the tube, the contents were transferred to tubes containing selective liquid media, EC, and BGB Broth medium. The EC Broth tubes were incubated at 44 °C as well and the BGB tubes were incubated at 37 °C with gas production assessed after 24 and 48 hours and compared with the Most Probable Number (MPN) table (Urooj *et al.*, 2018).


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### *Ethical considerations*

This study was approved at Ethics Committee of Shahid Sadoughi University of Medical Sciences. (ID: IR.SSU.SPH.REC.1402.015)

### *Data analysis*

SPSS software version 26 was used for data analysis. The parameters of total coliforms, fecal coliforms and fungal contamination levels were assessed. To investigate differences in categorical distributions, Chi-square test (Fungal species) and to compare differences in mean colony counts between two preparation methods, Mann-Whitney test was used.

## Results

### *Fungal quantity and quality identification*

**Table 1** shows results of fungi colony enumeration in *Ab-chubak* prepared by boiling and manual methods.

**Table 1.** Fungal colony enumeration as CFU/ml.

| Production method | Mean±SD | Min | Max | Median |
|---|---|---|---|---|
| Manual | 46.63±53.90 | 0 | 240 | 42 |
| Boiling | 30.98±45.66 | 0 | 200 | 15 |

As shown in **Table 1**, mean numbers of fungal colonies in manual and boiling methods were 46.63±53.90 CFU/ml and 30.98±45.66 CFU/ml, respectively. Results of Mann-Whitney test indicated a significant difference in mean number of fungal colonies between two *Ab-chubak* preparation methods ($P$=0.04). In other words, the abundance of fungal numbers was not the same in two preparation methods.

The morphology of four fungal species identified in this study is shown in **Figure 2** and the Frequencies of fungal types are shown in **Figure 3**.

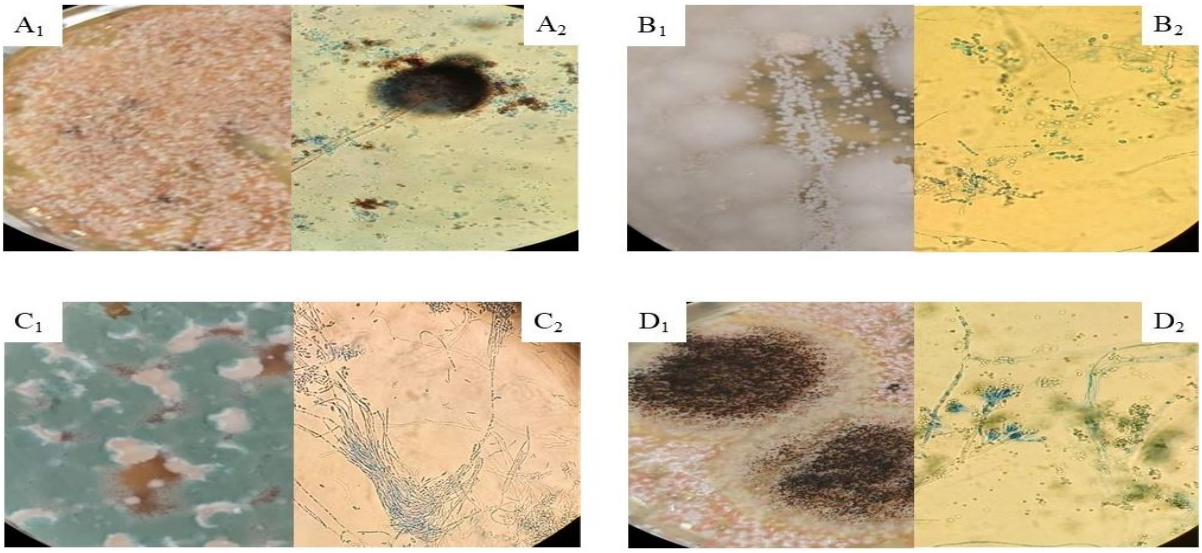


**Figure 2.** The morphological images of grown fungi in the present study (A*: Aspergillus*, B: *Yeast*, C: *Geotrichum*, D: *Penicillium*)



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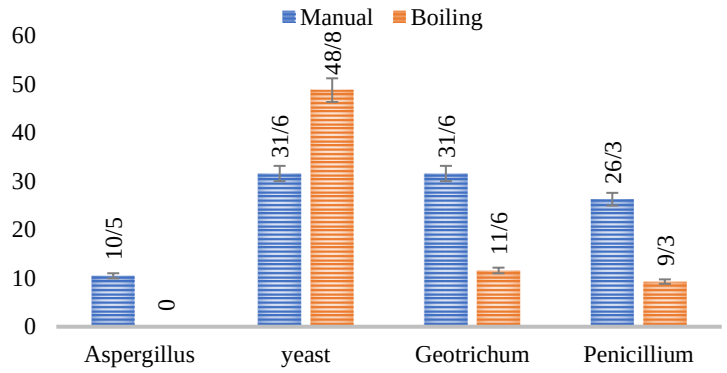


**Figure 3.** Fungal type percents in examinated Ab-chubak.

As shown in **Figure 2**; the prevalence of *Geotrichum, Penicilium,* and *Aspergillus* was 31.6, 26.3 and 10.5%, in the manual method, while *Geotrichum* and *Penicilium* were found at a prevalence of 11.6 and 9.3% in boiling methods. However, *Aspergillus* was not detected in *Ab-chubak* prepared by the last method. Overall, manual method showed a higher prevalence of *Geotrichum*, *Penicillium*, and *Aspergillus* compared to boiling method, whereas *yeasts* were more prevalent in boiling method. The results of chi-square test also showed a significant difference ($P$=0.002). In other words, the morphology of investigated fungi differed between two *Ab-chubak* preparation methods.

***Total and fecal coliforms enumeration***

**Table 3** shows the average total and fecal coliforms in *Ab-chubak* produced using boiling and manual methods.

**Table 3.** Total and Fecal coliforms in Ab-chubak samples (MPN/100 ml).

| Bacterial indicator | Mean±SD | Min | Max |
|---|---|---|---|
| Total coliform Manual | 392.52±1065.68 | 0 | 3600 |
| Boiling | 508.25±1053.62 | 0 | 3700 |
| Fecal coliform Manual | 308.31±878.24 | 0 | 3600 |
| Boiling | 434.55±899.48 | 0 | 3200 |

According to **Table 3**, the mean of total coliform in manual and boiling preparation methods were 392.52±1065.68 and 508.25±1053.62 MPN/100 ml, respectively. Similarly, fecal coliform counts were 308.31±878.24 and 434.55±899.48 MPN/100 ml, respectively. However, results of statistical analysis using Mann-Whitney test indicated that mean values of these two parameters ($P$=0.94 for TC and 0.66 for FC) did not differ significantly between two *Ab-chubak* preparation methods.

## Discussion

Analysis of microorganisms gives vital data on cleanliness of food, its expiration date, and possible health hazards (Lima *et al.*, 2023). Detecting pathogens, spoilage microbes, and other contaminants in complicated food matrices is crucial (Ferone *et al.*, 2020). This study examined microbial parameters in Tahini production units and identified three different types of *yeast* fungi including *yeast, Aspergillus, Geotrichum*, and *Penicillium*. This is consistent with a study conducted by Mahdian Yazdi *et al*. on Halva Tahini, which reported that among 60 samples (halva ardeh), three were contaminated with *molds*, one with *yeast*, four with *Escherichia coli*, and one with *Enterobacteriaceae* (Yazdi *et al.*, 2018).The results of fungal counts indicated that 31.6% of manually prepared samples were contaminated with *yeast*, whereas 48.8% of samples prepared by boiling showed *yeast* contamination. Similar results were obtained from conducted studies by Sengun *et al*. and Kotzekidou; $1.5\times10^2$ and $4.9\times10^3$ CFU/g, respectively (Kotzekidou, 1998, Sengun *et al.*, 2005). Additionally, a study by Kilci *et al.* on


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various types of Halva Tahini revealed that microbiological analyses for *yeast* and *mold* contamination showed an average of 10 CFU/g, 90 CFU/g in hazelnut Halva Tahini, 45 CFU/g in carob Halva Tahini, and 25 CFU/g in vanilla Halva Tahini (Kilci and Çetin, 2022).

This difference may be attributed to characteristics of *yeasts*, such as their mesophilic and thermophilic properties, which make them more resistant compared to other fungi like *molds*. Additionally, aerobic conditions may inhibit *mold* growth, which could explain the higher prevalence of *yeasts* in boiling method compared to manual method (Sengun *et al.*, 2005). Another reason for the identification of *yeast* in boiling samples could be related to the storage of *Ab-chubak* in refrigerator after boiling method. This may allow for the transfer of *yeast* contamination through the air inside the refrigerator.

Regarding *Geotrichum* and *Aspergillus* fungi, results indicated that their prevalence in manual samples was 31.6% and 10.5%, respectively, whereas, in boiling method, *Geotrichum* was present at 11.6% and *Aspergillus* was not detected. Manual method showed a higher prevalence of *Geotrichum*, *Penicillium*, and *Aspergillus* compared to boiling method, while *yeasts* were more prevalent.

In the study by Kahraman on Halva Tahini samples, it was found that 36.66% of samples had poor quality based on microbiological and chemical standards recommended by Turkish Food Codex. The microbiological quality criteria included aerobic bacteria, *Staphylococcus aureus*, coliforms, *Escherichia coli*, *molds*, *yeasts*, *Salmonella* species, and *staphylococcal enterotoxins*. Chemical quality criteria included sesame oil, protein, moisture, ash content, acidity, total sugar content, and peroxide levels. The predominant level of contamination was 10.38% for *molds*, which was lower than the contamination level for *yeasts* at 38.20%. This could be attributed to factors such as inadequate hygiene of raw materials, production area, and sample storage conditions (Kahraman *et al.*, 2010).

In this study, the mean of number of TC was 392.52±1065.68 and 508.25±1053.62 MPN/100 ml for manual and boiling methods, respectively. Additionally, the number of FC in mentioned samples was 308.31±878.24 and 434.55±899.48 MPN/100 ml, respectively. In other words, TC and FC counts were approximately similar in two methods, with no significant differences. Given the relatively long storage duration, one reason for the higher coliform counts could be attributed to the storage of these samples in the refrigerator after boiling. This storage method may allow for the transfer of coliform contamination through secondary contamination. Another reason could be related to secondary contamination via human preparation or the preserved dishes that transferred bacterial contamination.

Most similar studies have mentioned that Halva may become contaminated with various microorganisms due to direct or indirect contact with external sources of contamination during production (Abu-Jdayil *et al.*, 2002). the results of the present study, indicate that such contamination may result from the microbial flora of raw materials, such as *Ab-chubak*, insufficient thermal treatment, and inadequate personal hygiene during production (Aydin and Başaran, 2018).

In a study conducted by Karam *et al*. multiple outbreaks of *S. bovismorbificans*, *S. Montevideo*, and *S. Mbandaka* infection were related to Tahini consumption that were reported in the United States. Moreover, Escherichia coli was found in 17% of Tahini samples in Lebanon (Karam, 2010).

In a study conducted by Aydın *et al.* on Tahini halva samples, the results showed that coliforms and *Escherichia coli* were found at acceptable levels (28 MPN/g) (Aydin and Başaran, 2018). Sengun *et al*. reported coliforms levels of 41 CFU/g, and Kotzekidou reported 30 CFU/g, which are consistent with the findings of the present study regarding the identification of coliforms and *Escherichia coli* (Kotzekidou, 1998, Sengun *et al.*, 2005).

In a study conducted by Eshaghi on Halva Tahini in Yazd and Ardakan, it was found that 8.3% of Halva Tahini samples from Ardakan and 12.5% of samples from Yazd were contaminated



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with *Escherichia coli* (Eshaghi, 2010). Since the presence of *Escherichia coli* in food indicates significant fecal contamination, inadequate personal hygiene may be a significant factor contributing to *Escherichia coli* contamination in Halva (Namiki, 1995). However, the results of studies conducted on Halva Tahini are promising, in the sense that the process of preparing Halva Tahini can reduce the bacterial indicators identified in Abchoubak, but it is still recommended to monitor this product regarding fungal surveillance.

Despite valuable findings of this study, a few limitations should be acknowledged. Molecular identification and mycotoxin analysis were notable limitations of this study, and their investigation is recommended for future research.

## Conclusion

The present study underscores the need for microbiological quality control in *Ab-chubak*, a key additive in Halva Tahini production. The findings highlighted the presence of opportunistic fungal species and fecal coliforms, indicating potential health risks. Given the absence of regulatory standards for *Ab-chubak*, establishing effective contamination control measures is crucial. Implementing strict hygiene protocols, heat treatments, and improved monitoring practices across production and distribution can significantly reduce microbial contamination. These measurements will enhance food safety, minimize health risks, and ensure the hygienic quality of *Ab-chubak* in food applications.

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## Authors' contributions

Design study was conducted by Teimouri F, Sadeghizadeh-Yazdi J. Experimentations were performed by Paydar A, Shiranian M, Naderipour Z. Data analysis was conducted by Jambarsang S. The draft manuscript was written by Kamali H and Teimouri F. All authors read and approved the final manuscript.

## Conflict of interest

No potential conflict of interest is reported by the author(s).

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