Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

First Report of *Hanseniaspora guilliermondii* as a Spoilage Yeast in Greek Yogurt with Mixed Berries in Egypt

Rabee A. Ombarak*

Department of Food Hygiene and Control, Faculty of Veterinary Medicine 32897, University of Sadat City, Egypt.

Abstract

THE purpose of this study was to investigate the causative agent of a spoilage incident of Greek yogurt with mixed berries produced by one of the largest dairy manufacturing companies in Egypt. For this purpose, 5 samples from the affected batch were randomly collected and tested for sulfite-reducing bacteria growing under anaerobic conditions, coliforms, yeasts, and molds. The results of enumeration of sulfite-reducing bacteria and coliforms were below the detection limit, while yeasts and molds agar plates showed growth of yeast.

From each sample, a yeast isolate was selected and identified by 5.8S-ITS rRNA analysis and sequencing. The yeast species was identified as *Hanseniaspora guilliermondii* in all the examined samples. This study is the first to report the association of *H. guilliermondii* with the spoilage of yogurt. This study highlights the importance of controlling non-Saccharomyces yeasts in yogurt production. Further research on the specific spoilage mechanisms of *H. guilliermondii* in dairy products is recommended.

Key words: Greek Yogurt; spoilage yeast; *Hanseniaspora guilliermondii*, Egyptian dairy industry.

Introduction

Fermented milk and milk-based products of different formulations in different names are popular throughout the world for their taste as well as health benefits.

One of the fermented milks that their demand has been greatly increased worldwide is Greek yogurt due to its health benefits, adaptability, and the growing popularity of low-sugar, high-protein diets. Greek yogurt is a traditional yogurt that is strained to remove extra whey, giving a thicker consistency and a concentrated nutritional profile. Greek yogurt is a nutrient-dense dairy product with almost twice the protein level of traditional yogurt, it is a great source of high-quality protein [1].

Greek yogurt also contains probiotics, which are good bacteria that maintain gut microbiota and enhance digestive health, and is high in calcium, which supports bone health [2]. It is a good choice for people on low-carb or diabetic-friendly diets because it contains fewer carbohydrates than conventional yogurt [3].

Fermented dairy products are generally considered microbiologically stable because they are most commonly produced from heat-treated milk, contain competitive microbiota, which acidify the product by producing organic acids and stored refrigerated. However, they are susceptible to spoilage due to fungal (i.e., yeasts and molds) contamination, since many fungal species can flourish under low pH and low temperature environments [4], resulting in visual defects, unpleasant sensory characteristics and physical deterioration of the product [5].

Fungal contamination of fermented dairy products may originate from the production environment, frequently from surfaces, or from the raw ingredients included after fermentation, such as fruit preparations and other additives [5, 12].

Spoilage fungi isolated from yogurt and cultured dairy products represent a broad diversity of yeasts and molds with yeasts often cited as the primary spoilage organism in these products [5].

*Corresponding authors: Rabee A. Ombarak, E-mail: rabee.alhossiny@vet.usc.edu.eg Tel.: +201012486590
(Received 14 December 2024, accepted 02 February 2025)
DOI: 10.21608/EJVS.2025.344310.2559

Yeast spoilage of fermented dairy products presents issues for the dairy sector, resulting in economic losses from product wastage. Worldwide, around 11 to 25% of dairy products are wasted, varying by geography [6], with a significant portion lost due to fungal deterioration.

In Egypt, dairy production is a key agricultural sector, yet spoilage incidents threaten product quality and consumer trust.

This study aimed to investigate the causative agent of a spoilage incident of Egyptian Greek yogurt with mixed berries at one of the biggest dairy manufacturing companies in Egypt.

Material and Methods

Collection of samples

From a batch (50,000 cups, 180 grams each) of Greek yogurt with mixed berries, manufactured by one of the the biggest dairy manufacturing companies in Egypt, showing signs of spoilage (as bulging of the lid or gas comes out when yogurt cups lid is removed, off-odors [yeasty odors] and body defects) before its distribution, 5 samples were randomly collected and immediately transported to the laboratory at 4°C and analyzed on the same day to investigate the spoilage causative agent.

Identification of the spoilage causative microorganism

Each sample of the Greek yogurt with mixed berries were thoroughly mixed and 25 grams of each sample were aseptically homogenized with 225 ml of sterile maximum recovery diluent and tenfold serial dilutions were prepared for determination of the causative spoilage microorganism.

All samples underwent detection and enumeration of sulfite-reducing bacteria growing under anaerobic conditions, coliforms, yeasts and molds as follows:

Detection and enumeration of sulfite-reducing bacteria

Sulfite-reducing bacteria growing under anaerobic conditions were tested according to ISO 15213 [7]. Briefly, 1 ml of each initial dilution were transferred into Petri dishes and approximately 15 ml of Iron Sulfite Agar (Merck) previously cooled to 44 °C to 47 °C, were poured into each Petri dish, then carefully mixed and allowed to solidify, and incubated under anaerobic conditions at 50 ºC for 48 h and 37 ºC for 48 h, for thermophilic and for mesophilic bacteria, respectively.

Detection and enumeration of coliform

The colony-count technique at 37 °C was used as described elsewhere [8]. Briefly, 1 ml of the tenfold serial dilution of each sample was transferred into Petri dishes and approximately 15 ml of crystal violet neutral red bile lactose (VRBL) agar medium (Oxoid, UK), previously cooled to 44 °C to 47 °C, were poured into each Petri dish, then carefully mixed and allowed to solidify, and incubated at 44ºC for 24 h.

Detection and enumeration of yeasts and molds

Detection and enumeration of yeasts and molds was done according to ISO 6611 [9]. Briefly, the samples dilutions were inoculated on yeast-extract-glucose- chloramphenicol agar (YGC) (Merck, Germany) and yeast and/or mould colonies growing on the plates were counted after 5 days of incubation at 25 ºC.

The colonies on plates showing growth were identified based on morphological properties and subjected to molecular identification using 5.8S-ITS rRNA analysis and sequencing as described below.

Identification of yeast isolates by 5.8S-ITS rRNA analysis and sequencing

DNA extraction was performed using QIAamp DNA Mini Kit (Qiagen®) (Qiagen, Germany, GmbH) following the manufacturer's recommendations. The extracted DNA was used as a template for PCR as described by Tarini et al., [10] using primers shown in table 1.

Briefly, PCR was performed in 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer (20 pmol concentration), 4.5 µl of sterile distilled water, and 6 µl of DNA template. The PCR condition was as follows: 94°C for 5 min; 35 cycles of 94°C for 30 sec, 56°C for 40 sec, 72°C for 45 sec, and a final extension step at 72˚C for 10 min. The amplicons were separated by electrophoresis on 1.5% agarose gel. Further, these amplicons were used as templates for the sequencing reaction.

One strain from each sample was chosen for sequencing. PCR products of selected strains were cleaned with a QIAquick Gel extraction kit (Qiagen, Germany) and sequenced on both DNA strands by using ITS1 and ITS4 primers and the BigDye Terminator v 3.1 sequencing standard kit (ThermoFisher Scientific) following the manufacturer's instructions and subjected to sequencing in an Applied Biosystems 3500 Genetic analyzer kit (ThermoFisher Scientific)**.**

Sequence comparisons were performed online using the basic local alignment search tool (BLAST) program available at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/blast).

Results

Identification of the causative spoilage microorganism

The results of detection and enumeration of sulfite-reducing bacteria growing under anaerobic conditions and coliforms were below the detection limit in the examined samples (<10 cfu/g) On the other hand, yeasts and molds agar plates showed growth of yeast colonies (Table 2).

Identification of yeast isolates

Based on colony morphology and microscopic observation, The isolates exhibited white creamy colonies with smooth surfaces with circular margins and the isolates were identified as budding yeast under microscope.

The results of 5.8S-ITS rRNA analysis (Fig. 1) and sequencing showed that the obtained yeast isolates were identified as *Hanseniaspora guilliermondii* in all of the examined samples (GenBank accession number: PQ655481).

DISCUSSION

Even though the additional processing steps for Greek yogurt generally do not directly expose the product to the processing environment, control measures are still necessary to prevent fungal contamination during production, particularly during filling and packaging when the product is exposed to the processing environment.

Sulfite-reducing bacteria and/or coliforms are usually alleged for yogurt spoilage; however, they were not the causative agent of spoilage incident that has been investigated in the current study.

The predominant yeast species that frequently contaminate fermented dairy products are Candida spp., *Torulaspora delbrueckii*, *Saccharomyces cerevisiae*, Rhodotorula spp., *Debaryomyces hansenii*, *Kluyveromyces marxianus*, and *Yarrowia lipolytica* [4, 11-12] (). However, in the current study none of them was the cause of the spoilage incident.

The combined use of colony morphology and microscopic observation and molecular methods in the current study have led to the identification of the yeast species. This study is the first to report the association of *H. guilliermondii* with the spoilage of yogurt.

Hanseniaspora, are yeasts mainly found mainly in soil, on fruits and trees and in spoiled foods and beverages, and there are several species in the genus Hanseniaspora which are physiologically very similar [13].

H. guilliermondii is a widespread species that is mostly linked to plants and a variety of fruits [14-15]. It was also reported as a prevalent species in early stages of cocoa bean fermentations in Indonesia and in West Africa [16-18]. Arroyo-López et al., [19], by using molecular methods, detected occurrence of *H. guilliermondii* in processed black table olives.

H. guilliermondii, is known for its fermentative abilities and production of enzymes like β-glucosidase, may contribute to flavor changes and spoilage. *H. guilliermondii* is one of the prevailing apiculate yeast species on wine grapes [20].

In Egypt, where fruit inclusions are increasingly used in yogurt, ingredient screening is critical. *H. guilliermondii*'s fermentative metabolism may explain gas production and off-odors. Even low initial contamination ($<10^2$ CFU/g) can lead to spoilage during cold storage, underscoring the need for improved hygiene and bioprotective cultures [12].

Consequently, we believe that the berry ingredients likely introduced these yeasts to the Greek yoghurt examined in our study, which then proliferated during storage especially that fungal spoilage can occurs throughout the product's shelf life and may arise from minimal initial contamination levels.

Dairy producers may find it challenging to identify quality flaws in a particular batch prior to the expiration of its shelf life. Therefore, it is critical to comprehend and forecast how various factors, including storage temperature, duration, and the existence of food cultures with bioprotective properties, affect the quality of the final product [12].

CONCLUSION

In conclusion, this study identifies *H. guilliermondii* as a spoilage agent in Egyptian Greek yogurt, marking its first association with dairy products. Molecular methods were essential for accurate identification, highlighting their role in quality assurance. To mitigate risks, dairy manufacturers should enhance fruit ingredient sterilization and monitor cold chain integrity. Further research on *H. guilliermondii's* spoilage mechanisms in dairy products is. recommended.

Acknowledgments

Not applicable.

Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

Not applicable. In this study, no humans or animals were involved.

TABLE 1. Primers sequences, target gene, amplicon size

Target gene	Primers sequences (5'→3')	Amplified segment (bp)
ITS	ITS1: TCCGTAGGTGAACCTGCGG	Variable
	ITS4: TCCTCCGCTTATTGATATGC	

TABLE 2. Enumeration of sulfite-reducing bacteria, coliforms, yeasts and molds in spoiled Greek yogurt with mixed berries samples

Tested microorganism	Results (CFU/g)
Sulfite-reducing bacteria	<10
Coliforms	<10
Yeasts	$>3x10^4$
Molds	<10

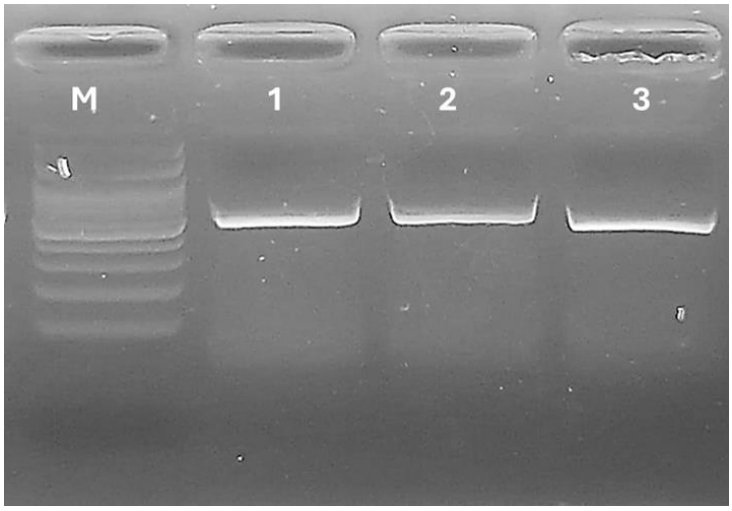

Fig 1. Representative results of PCR amplification of 5.8S-ITS. Lanes 1 to 3: yeast isolates, M: Marker (100 bp ladder)

References

1. El-Abbadi N.H., Dao M.C. and Meydani S.N., Yogurt: Role in Healthy and Active Aging, *American Journal of Clinical Nutrition*, **99**(5), 1263S–1270S (2014). https://doi.org/10.3945/ajcn.113.073957
2. Anbari K., Firouzi M. and Abbaszadeh S., Probiotics and Gastrointestinal Diseases: A Promising Complementary Medicine Resource for Treatment of Gastrointestinal Disorders and Diseases, *Journal of Pharmacy and Pharmacognosy Research*, **7**, 194 (2019).
3. Vieira T.C., Pinto V.R.A., Rocha F., Pires A.C.S., Minim V.P.R. and Vidigal M.C.T.R., New Insights into Perceptions of Technology Claims in Greek-Style Yogurt: A View in the COVID-19 Pandemic, *Food Research International*, **161**, 111822 (2022). https://doi.org/10.1016/j.foodres.2022.111822
4. Martin N.H., Snyder A. and Wiedmann M., Spoilage Mold in Dairy Products, *Encyclopedia of Dairy Science*, 607–610 (2022). https://doi.org/10.1016/b978-0-08-100596-5.23017-5
5. Ledenbach L.H. and Marshall R.T., Microbiological Spoilage of Dairy Products, In: Sperber W.H., Doyle M.P. (eds.), *Compendium of the Microbiological Spoilage of Foods and Beverages*, 41–67, Springer, New York (2009). https://doi.org/10.1007/978-1-4419-0826-1_2
6. FAO, Global Food Losses and Food Waste: Extent, Causes, and Prevention, *Food and Agriculture Organization of the United Nations*, (2011).
7. ISO, Microbiology of Food and Animal Feeding Stuffs - Horizontal Method for the Enumeration of Sulphite-Reducing Bacteria Growing Under Anaerobic Conditions, ISO 15213, *International Organization for Standardization*, Geneva (2003).
8. ISO, Microbiology of Food and Animal Feeding Stuffs - Horizontal Method for the Enumeration of Coliforms - Colony-Count Technique, ISO 4832,

International Organization for Standardization, Geneva (2006).

9. ISO, Milk and Milk Products – Enumeration of Colony-Forming Units of Yeasts and/or Molds – Colony-Count Technique at 25 Degrees C, ISO 6611, *International Organization for Standardization*, Geneva (2004).
10. Tarini N.M.A., Wahid M.H., Ibrahim F., Yasmon A. and Djauzi S., Development of Multiplex-PCR Assay for Rapid Detection of *Candida* spp., *Medical Journal of Indonesia*, **19**(2), 83 (2010). https://doi.org/10.13181/mji.v19i2.387
11. Viljoen B.C., The Interaction Between Yeasts and Bacteria in Dairy Environments, *International Journal of Food Microbiology*, **69**(1-2), 37–44 (2001). https://doi.org/10.1016/S0168-1605(01)00570-0
12. Garnier L., Valence F. and Mounier J., Diversity and Control of Spoilage Fungi in Dairy Products: An Update, *Microorganisms*, **5**(3), 42 (2017). https://doi.org/10.3390/microorganisms5030042
13. López S., Mateo J.J. and Maicas S., Characterisation of *Hanseniaspora* Isolates with Potential Aroma-Enhancing Properties in Muscat Wines, *South African Journal of Enology and Viticulture*, **35**(2), 292–303 (2014). https://doi.org/10.21548/35-2-1018
14. Yurkov A., Yeasts in Forest Soils, In: Buzzini P., Lachance M.A., Yurkov A. (eds.), *Yeasts in Natural Ecosystems: Diversity*, 3–24, Springer, Cham (2017). https://doi.org/10.1007/978-3-319-62683-3_3
15. van Wyk N., Badura J., von Wallbrunn C. and Pretorius I.S., Exploring Future Applications of the Apiculate Yeast *Hanseniaspora*, *Critical Reviews in Biotechnology*, **44**(1), 100–119 (2023). https://doi.org/10.1080/07388551.2022.2136565
16. Ardhana M.M. and Fleet G.H., The Microbial Ecology of Cocoa Bean Fermentations in Indonesia, *International Journal of Food Microbiology*, **86**(1-2), 87–99 (2003). https://doi.org/10.1016/S0168-1605(03)00081-3
17. Jespersen L., Nielsen D.S., Hønholt S. and Jakobsen M., Occurrence and Diversity of Yeasts Involved in Fermentation of West African Cocoa Beans, *FEMS Yeast Research*, **5**(5), 441–453 (2005). https://doi.org/10.1016/j.femsyr.2004.11.002
18. Nielsen D.S., Teniola O.D., Ban-Koffi L., Owusu M., Andersson T.S. and Holzapfel W.H., The Microbiology of Ghanaian Cocoa Fermentations Analysed Using Culture-Dependent and Culture-Independent Methods, *International Journal of Food Microbiology*, **114**(2), 168–186 (2007). https://doi.org/10.1016/j.ijfoodmicro.2006.09.010
19. Arroyo-López F.N., Durán-Quintana M.C., Ruiz-Barba J.L., Querol A. and Garrido-Fernández A., Use of Molecular Methods for the Identification of Yeasts Associated with Table Olives, *Food Microbiology*, **23**(8), 791–796 (2006). https://doi.org/10.1016/j.fm.2006.02.008
20. Nisiotou A.A. and Nychas G.-J.E., Yeast Populations Residing on Healthy or *Botrytis*-Infected Grapes from a Vineyard in Greece, *Applied and Environmental Microbiology*, **73**(8), 2765–2778 (2007). https://doi.org/10.1128/AEM.01864-06

التقرير الأول عن *Hanseniaspora guilliermondii* كمسبب تلف في الزبادي اليوناني مع التوت المختلط في مصر

ربيع الحسيني امبارك *

قسم الرقابة الصحية على الأغذية، كلية الطب البيطري، جامعة مدينة السادات، مصر.

الملخص

كان الغرض من هذه الدراسة هو التحقيق في العامل المسبب لحادث تلف الزبادي اليوناني مع التوت المختلط الذي تنتجه واحدة من أكبر شركات تصنيع الألبان في مصر.

لهذا الغرض، تم جمع 5عينات من الدفعة المصابة بشكل عشوائي واختبارها بحثا عن البكتيريا المختزلة للكبريتيت التي تنمو في ظل الظروف اللاهوائية والقولونيات والخمائر والعفن .كانت نتائج تعداد البكتيريا المختزلة للكبريتيت والقولونيات أقل من حد الكشف، بينما أظهرت نتائج الكشف عن الخمائر والعفن نموا للخميرة .بعدها تم اختيار معزولة خميرة من كل عينة، وتحديدها من خلال تحليل وتسلسل 5.8S-ITS rRNA. تم تحديد أنواع الخميرة على أنها *Hanseniaspora guilliermondii* في جميع العينات التي تم فحصها .هذه الدراسة هي الأولى التي أبلغت عن ارتباط *H. guilliermondii* بتلف الزبادي .تسلط هذه الدراسة الضوء على أهمية التحكم في الخمائر غير الخميرة في إنتاج الزبادي .يوصى بإجراء مزيد من البحث حول آليات التلف المحددة ل *H. guilliermondii* في منتجات الألبان.

الكلمات الدالة: زبادي يوناني، خميرة *Hanseniaspora guilliermondii* ، صناعة الألبان المصرية.