

RESEARCH ARTICLE

Fungi associated with long-term stored beet thick juice: effect of temperature and biocide treatments

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Abstract

This study focuses on the storage of beet thick juice in hot-weather regions like Egypt, where maintaining lower storage temperatures is economically unfeasible and challenging, with the aim of enhancing white sugar yield and mitigating the effects of climate change. Fungal contamination during storage not only compromises the microbial quality of beet thick juice but also poses significant economic risks by reducing product purity and processing efficiency in the sugar industry.

This study utilized a pilot plant comprising twelve storage cylinders to store thick beet juice with total soluble solids (^oBrix) of 67, 68, and 69 at 15, 25, and 35 °C, respectively. Hop β -acids and KEOCID 310 biocides were used at 40 ppm, in addition to surface sealing by 25.0% NaOH with air removal.

The fungal count (CFUs) was assessed at each treatment. Twelve species of fungi from four genera were isolated and identified, yielding 2334 CFUs in total. *Acremonium strictum* was the predominant species, accounting for 16.1% of total fungi. *Aspergillus niger*, *A. flavus*, *A. ustus*, and *A. terreus* ranked the next, constituting 13.1 %, 11.2 %, 11.6 %, and 9.2 % of the total fungi, respectively. *Cladosporium cladosporioides* (6.9 % of total fungi), was also identified. *Penicillium aurantiogriseum*, *P. chrysogenum*, and *P. oxalicum* comprised 7.9 %, 4.4 %, and 4.9 % of total fungi, respectively.

The results indicated that 15 °C was the optimal temperature for preservation, as it yielded the lowest fungal count (375 CFU/mL), whereas 35 °C exhibited the highest overall fungal count (1199 CFU/mL). At each of the three storage temperatures—15, 25, and 35 °C—the control tanks exhibited higher CFUs compared to the treated tanks.

The CFUs in the control tanks exhibited a direct proportional increase with temperature, with 35 °C yielding the highest CFUs in comparison to 15 and 25 °C. Tanks containing hop β -acid demonstrated the highest antifungal activity at 15, 25, and 35 °C, as evidenced by the lowest fungal growth recorded at 45, 130, and 174 CFU/mL, respectively.

Keywords: Beet; Biocides; Fungi; Storage; Thick juice; Xerophiles.

Introduction

Fungi represent a highly diverse category of creatures, varying in size from minute unicellular yeasts to substantial macro fungi, exemplified by the familiar mushrooms and toadstools, including the largest fruiting body, the colossal puffball. Hawksworth (1991) asserts that over one million species remain undescribed. They are often found in a wide range of environments because they can colonize a wide range of substrates (Al-Bedak and Faysal 2024).

The term "Xerophilic" originates from Greek, indicating "dry loving". Pitt and Hocking (2009) categorize xerophilic fungi into two groups: moderate xerophiles and extreme xerophiles.

Extreme xerophilic filamentous fungi are either completely inhibited by increased water activity (a_w) or demonstrate markedly diminished growth rates.

Conversely, moderate xerophiles include *Paecilomyces variotii*, *Aspergillus pseudoglaucus*, *A. chevalieri*, *A. glaucus*, *A. montevidensis*, *A. ruber*, and *Penicillium* spp. (Pitt and Hocking 2009, Rico-Munoz *et al.* 2019).

Sugar beet thick juice is the concentrated juice obtained through evaporation during the sugar manufacturing process (Figure 1).

The concentration of total soluble solids is approximately 69 ^oBx, accompanied by a slightly alkaline pH of about 9.0 (Willems *et al.* 2003, Asadi 2006, Justé *et al.* 2008b).

Thick juice is typically stored for long durations between the beet processing campaign and final sugar crystallization. Its storage allows for flexible year-round sugar production. However, due to its high sugar content and relatively low water activity, it remains accountable to microbial contamination—particularly by osmotolerant fungi—if not stored under controlled temperature and hygienic conditions.

Maintaining appropriate storage parameters is essential to preserving juice quality and minimizing microbial spoilage. Prior research and industry practices indicate that the stability of thick juice is best maintained through the regulation of factors including solids content, pH, and temperature, as these elements directly influence microbial proliferation (Willems *et al.* 2003, Asadi 2006).

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Despite the implementation of effective storage techniques, thick juice continues to degrade due to microbial contamination. Key indicators of deterioration include a reduction in pH from 9 to 5-6 and an increase in reducing sugar concentration (Sargent *et al.* 1997, Willems *et al.* 2003), resulting in decreased sucrose production and subsequent economic loss.

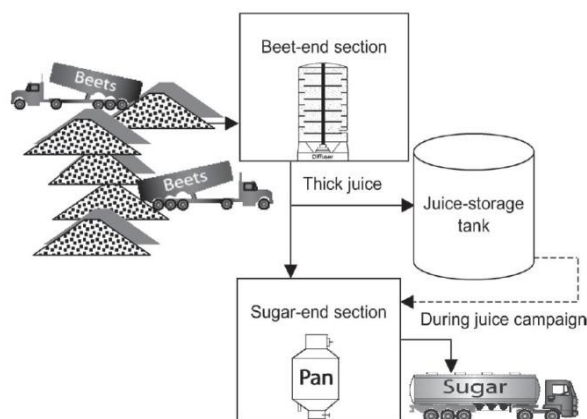


Figure 1. Flow diagram of beet thick juice storage (after Asadi (2006).

Regarding the hot climatic conditions in countries like Egypt, where ambient temperatures during storage can reach 35 °C or higher, understanding the temperature-dependent behavior of contaminant fungi is critical. Previous studies have mostly focused on bacterial contaminants or have evaluated microbial quality without linking it to specific storage conditions (Abdelhak *et al.* 2025).

There is a need for systematic evaluation of how varying storage temperatures affect fungal growth, species distribution, and subsequent implications for juice quality. Among the microbial threats, fungal contamination is particularly concerned due to its ability to survive in high-sugar, low-water activity environments and to grow under a broad range of temperatures. Some filamentous fungi not only degrade the quality of the stored juice by consuming sugars and producing off-flavors, but also pose a potential safety hazard through the production of mycotoxins. Despite its relevance, fungal spoilage in thick juice remains under-investigated, particularly in relation to storage temperature, a key factor that influences microbial growth and metabolic activity.

It is uncertain which particular fungi are responsible for the thick juice's degradation (Sargent *et al.* 1997, Willems *et al.* 2003). Sargent *et al.* (1997) linked pH drop to the mesophilic bacteria, while Willems *et al.* (2003) suggested a connection between thick juice degradation and the fastidious bacteria (FB), that grows on growth media enriched with blood instead of conventional bacterial plating media.

Many studies have examined the microbiota of sugar beets and the extraction juice (Hollaus and Klaushofer 1973, Bugbee *et al.* 1975, Belamri *et al.* 1991); however, very few investigations have documented the presence and growth of fungi in the highly concentrated thick juice (van der Poel *et al.* 1998, Willems *et al.* 2003). In line with the concept of chemical-free manufacturing, natural biocides have been increasingly investigated for their antimicrobial properties during thick juice preservation (Hein *et al.* 2006). Hop products (*Humulus lupulus* L.) were initially successfully used in the sugar industry in 1994 to inhibit microorganisms during beet juice extraction (Pollach *et al.* 1996).

These naturally-occurring hop biocides are harmless for both humans and animals, and they even have a crucial role in beer industry (Sakamoto and Konings 2003). Due to a lack of sufficient study on the environmental compatibility of thick juice preservation technologies, Egypt does not employ any strategy that extends the manufacturing season beyond the main season.

This study was directed towards the evaluation of various strategies for beet thick juice storage under the Egyptian environmental conditions. Fungi associated with the stored thick juice were isolated and identified. Additionally, the effect of natural and chemical biocides on the fungal growth was investigated. KEOCID 310, 10.0 % aqueous alkaline solution of hop- β -acid, and 25.0 % NaOH as preservatives, were used.

Materials and methods

Construction of storage pilot plant

A small-scale pilot plant was constructed to mimic the storage conditions of a larger system at the Alexandria Sugar Company in Egypt (Savola Foods, 30.819 N, 29.851 E). In the pilot plant, there was a system put in place to control the temperature at which the thick juice could be stored (15, 25, and 35 °C).

The system used chilled water circulation and embedded heaters to either cool or heat the stored thick juice according to the setpoint that was modified. Eighty liters of beet thick juice could be stored in the twelve double-walled stainless steel tanks of the system (Figure 1). Each tank had three sample taps arranged vertically. A 50 mm-thick jacket ringed the tank, allowing it to be heated or cooled by circulating water.

The inner cylinder of the juice was covered by a water jacket, and 100 mm of rock wool insulation encased it. A differential controller is used to adjust the temperature of the tank based on real-time data transmitted by an obstructed temperature sensor.

Design and conditions of the long-term storage experiment

Alexandria Sugar Company, Egypt, provided the beet thick juice with 70 °Brix from the manufacturing season 2023. The thick juice was collected in sterile 1,000-L polyethylene cubes following the evaporation stage, and the cubes were then kept until needed at 4 °C. When required, 25.0 % NaOH and sterile demineralized water were used to adjust the thick juice's pH and °Brix, respectively. The thick juice with °Brix values of 67, 68, and 69 was used for the storage experiment. In order to prevent sucrose nucleation, these circumstances kept the thick juice steady at a supersaturation coefficient of less than 1.0 (Asadi 2006, Schrevel 2009).

In addition to a control tank, three distinct treatments were utilized at each temperature group of 15, 25, and 35 °C. In compliance with industry standards (Willems *et al.* 2003, Beddie *et al.* 2004), a 10 % aqueous alkaline solution of Betastab® XL (Hop-β-acids), as well as KEOCID 310 (sodium dimethyldithiocarbamate), were applied to the thick juice at 40 ppm, as representatives of natural and chemical biocides, respectively (Pollach *et al.* 1996, Hein *et al.* 2002, Pollach *et al.* 2002, Willems *et al.* 2003, Justé *et al.* 2008a). The final treatment involved the physical removal of air using a blower and a microbiological filter affixed to one opening of the tank lid, in conjunction with the application of a 25% sodium hydroxide solution over the juice surface (Figure 2).

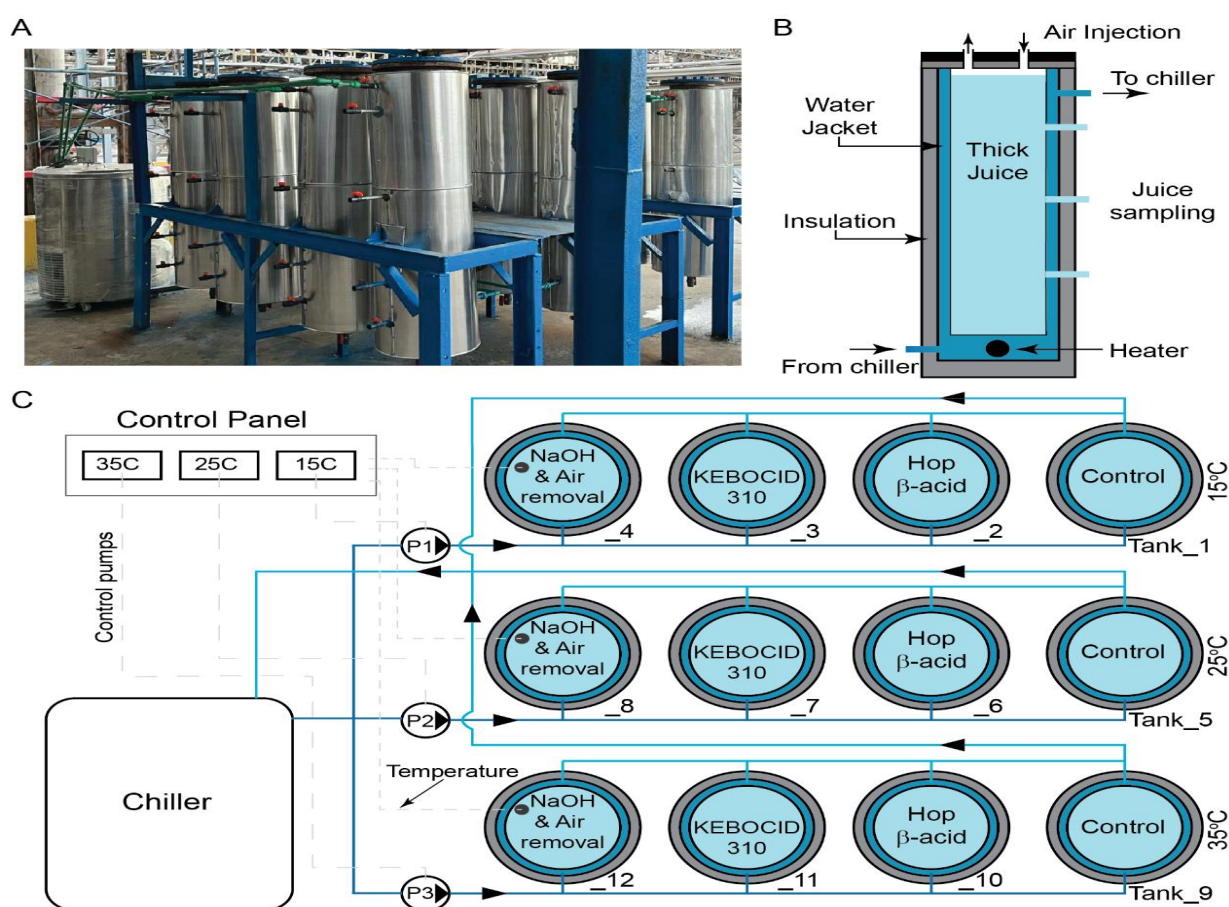


Figure 2. Schematic diagram of (A) Site image of the twelve tanks storing the beet thick juice. (B) hydraulic and the control circuit of the pilot plant consisted of twelve tanks used for storage of beet thick juice at 15, 25, and 35 °C. T1, T5, and T9 = Control; T2, T6, and T10 = tanks treated with Hop β-acid biocide; T3, T7, and T11 = tanks treated with KEOCID 310 biocide; T4, T8, and T12 = tanks treated with NaOH and air removal.

Sampling

Every 30 days of storage, samples of the stored thick juice were taken from each storage tank. All samples have been collected from the three taps along each storage tank in sterile glass bottles, and the samples were promptly brought to the laboratory for fungal isolation.

Isolation and identification of fungi

Using the dilution plate method (Warcup 1955), each of the 9 mm-diameter Petri dishes with Czapek's-Dox agar received 2.0 mL of the appropriate juice dilution. The isolation medium contained (g/L): Sucrose, 30; NaNO₃, 2; K₂HPO₄, 1.0; KCl, 0.5; MgSO₄, 0.5; ZnSO₄, 0.01; CuSO₄, 0.005; agar, 20. Rose Bengal (50 mg/L) and

chloramphenicol (250 mg/L) were added to suppress the growth of bacteria and to restrict the fungal colonies which facilitate the isolation of slow-growing fungi (Smith and Dawson 1944, Ismail et al. 2017). After that, the plates were incubated for 7 days at 25 °C. In each sample, the number of developed colonies was counted together with the colony forming units (CFU/mL) in each mL of the thick juice. The identification of fungal genera and species was based on macroscopic and microscopic features following the keys and descriptions illustrated by Raper and Fennell (1965) for *Aspergillus* species, Pitt (1979) for *Penicillium* species, and Moubasher (1993) for fungi in general.

In vitro evaluation of biocides activity on the proliferation of fungi

The well diffusion method (Magaldi et al. 2004) was employed to determine the inhibition zone of the two commercial biocides (hop-β-acid and KEOCID 310) against the tested fungal strains. Concentration of 40 ppm was produced from each biocide using distilled water. After cultivating the examined fungal strains in Czapek's broth medium, each Petri dish was separately inoculated with 1.0 mL of spore suspension (1.5×10^8 spores/mL). Wells with a diameter of 5 mm were created in the agar medium, and each well was separately filled with 50 µL of the biocide concentrations under examination. The plates were then incubated for 72 hours at 25 ± 1 °C. Following incubation, the suppression of fungal growth was measured as clear zones in millimeters. Three repetitions of each test were conducted.

Results

Isolation and identification of fungi from the stored beet thick juice

The present study's results showed that twelve fungal species representing four genera were isolated and identified, and they had 2334 CFUs overall. *Aspergillus* was the most common genus comprising 59.5 % of total fungi. It was represented by seven species, the most prevalent of which were *Aspergillus niger*, *A. flavus*, *A. ustus*, and *A. terreus* encountered 304, 263, 273, and 216 CFUs, and comprised 13.1, 11.2, 11.6, and 9.2 % of total fungi, respectively. *Penicillium* was the runner of *Aspergillus* constituting 17.0 % of total fungi followed by *Acremonium* (16.1 % of total fungi), and *Cladosporium* which encountered 6.9 % of total fungi (Table 1; Figure 3). *Penicillium* was represented by three species, namely *P. aurantiogriseum*, *P. chrysogenum*, and *P. oxalicum*. They comprised 7.9, 4.4, and 4.9 % of total fungi, respectively. *Acremonium strictum* was the most prevalent species with 376 CFUs and comprised 16.1 % of total fungi (Table 1; Figure 4). The results of this study showed that, for beet thick juice, 15 °C was the best temperature to keep it since it had the lowest fungal CFUs (375), while 35 °C showed the highest overall fungal count (1199). At each of the three storage temperatures—15, 25, and 35 °C—the control tanks (C1, C5, and C9) had greater CFUs than the treated tanks. Moreover, the CFUs in the control tanks increased in direct proportion to the temperature, with 35 °C showing the highest CFUs compared to 15 and 25 °C. Tanks with the biocide hop β-acid (C2, C6, and C10) exhibited the strongest antifungal effect at 15, 25, and 35 °C, since the lowest fungal growth was seen at 45, 130, and 174 CFUs, respectively (Table 1)

Table 1. List of fungal genera and species isolated from the beet thick juice stored at 15, 25 and 35 °C.

Fungal species	15 °C				25 °C				35 °C				Gross total CFUs	%Gross total
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12		
<i>Acremonium strictum</i>	20	11	14	11	30	30	41	24	66	38	72	19	376	16.1
<i>Aspergillus</i>	115	27	40	27	235	91	68	91	318	119	125	133	1389	59.5
<i>A. brasiliensis</i>	7	1	0	0	20	0	0	1	50	0	3	0	82	3.5
<i>A. flavus</i>	15	3	7	1	45	25	17	9	43	30	36	32	263	11.2
<i>A. fumigatus</i>	12	5	2	4	25	0	2	8	36	9	0	3	106	4.5
<i>A. niger</i>	24	6	8	8	44	24	14	30	50	22	33	41	304	13.1
<i>A. terreus</i>	16	5	9	3	28	19	14	17	45	22	9	29	216	9.2
<i>A. ustus</i>	23	5	14	11	41	16	8	21	55	18	33	28	273	11.6
<i>A. versicolor</i>	18	2	0	0	32	7	13	5	39	18	11	0	145	6.2
<i>Cladosporium cladosporioides</i>	14	0	6	4	23	0	9	12	55	0	15	25	163	6.9
<i>Penicillium</i>	51	7	20	8	92	9	5	0	181	17	12	4	406	17.3
<i>P. aurantiogriseum</i>	19	6	17	7	37	6	4	0	63	14	12	0	185	7.9
<i>P. chrysogenum</i>	19	0	2	0	22	2	1	0	58	0	0	1	105	4.4
<i>P. oxalicum</i>	13	1	1	1	33	1	0	0	60	3	0	3	116	4.9
Total	200	45	80	50	380	130	123	127	620	174	224	181	2334	100
No. of genera (4)	375				760				1199					
No. of species (12)														

Control = C1, C5, and C9; Hop β-acid = C2, C6, and C10; KEOCID 310 = C3, C7, and C11; NaOH with air removal = C4, C8, and C12.

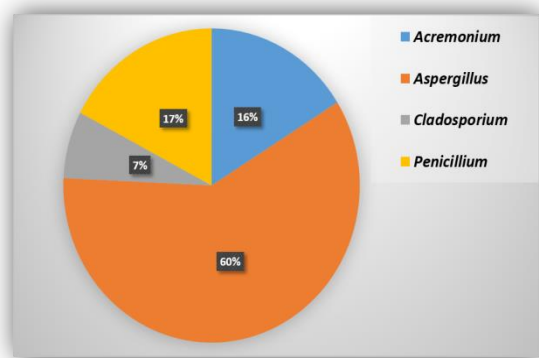


Figure 3. Percentage CFUs of the fungal genera isolated from the stored beet thick juice at 15, 25, and 35 °C

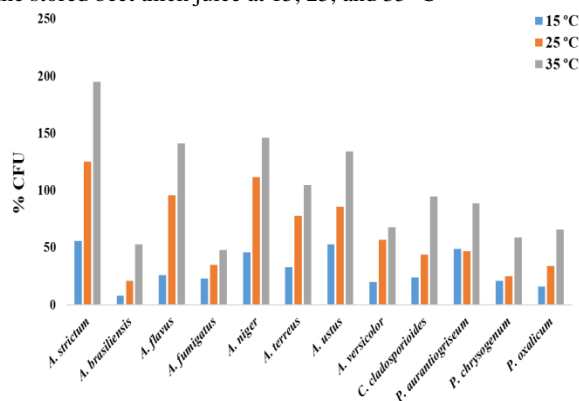


Figure 4. Percentage CFUs of the fungal species isolated from the stored beet thick juice at 15, 25, and 35 °C.

Acremonium strictum, *Aspergillus brasiliensis*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *A. ustus*, *A. versicolor*, *Cladosporium cladosporioides*, *Penicillium aurantiogriseum*, *P. chrysogenum*, and *P. oxalicum* were the fungal species that were isolated (Figure 5).

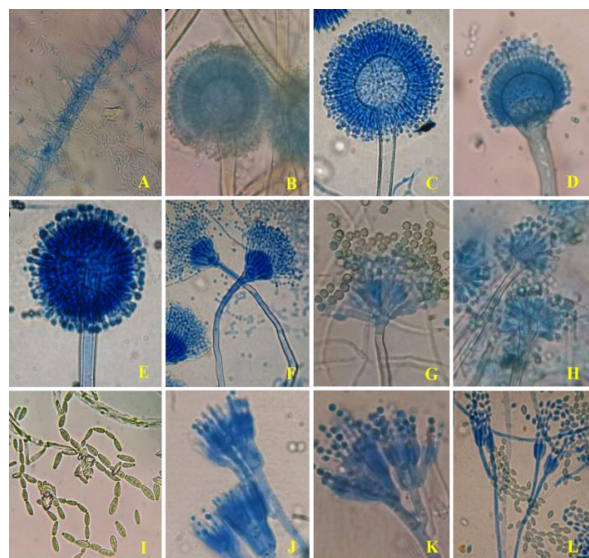


Figure 5. Fungal species recovered from the beet thick juice (A) *A. strictum* (B) *A. brasiliensis* (C) *A. flavus* (D) *A. fumigatus* (E) *A. niger* (F) *A. terreus* (G) *A. ustus* (H) *A. versicolor* (I) *C. cladosporioides* (J) *P. aurantiogriseum* (K) *P. chrysogenum* (L) *P. oxalicum*.

***In vitro* evaluation of biocides' activity on the fungal growth**

The current findings revealed that the chemical biocide (KEBOCID 310) shown more activity against all tested fungi compared to the natural biocide (Hop β -acid), which only affected *A. flavus* and *A. niger*. KEBOCID 310 completely inhibits the growth of all tested fungi, exhibiting clear zones of 90 mm (Table 2).

Table 2. Effect of 40 ppm addition of Hop- β acid and KEBOCID 310 biocides on the growth of fungal species isolated from the stored beet thick juice.

Fungal species	Hop- β acid (40 ppm)	KEBOCID 310 (40 ppm)
<i>Acremonium strictum</i>	0.0	90
<i>Aspergillus brasiliensis</i>	0.0	90
<i>A. flavus</i>	30	90
<i>A. fumigatus</i>	0.0	90
<i>A. niger</i>	30	90
<i>A. terreus</i>	0.0	90
<i>A. ustus</i>	0.0	90
<i>A. versicolor</i>	0.0	90
<i>Cladosporium cladosporioides</i>	0.0	90
<i>Penicillium aurantiogriseum</i>	0.0	90
<i>P. chrysogenum</i>	0.0	90
<i>P. oxalicum</i>	0.0	90

Discussion

During prolonged storage, sugar beet thick juice is susceptible to microbiological deterioration, especially due to osmophilic fungi. These microorganisms flourish in high-sugar, low-water activity environments, rendering thick juice a viable platform for their gradual yet persistent proliferation under inadequate storage conditions.

Fungal contamination may result in various quality concerns. Initially, it induces metabolic alterations in the juice, leading to the degradation of sugars into undesired byproducts such as alcohols and organic acids, which produce undesirable off-flavors and odors. Secondly, specific fungi can generate mycotoxins, which present significant detrimental effects and may render the product unsuitable for food preparation.

The current study's findings demonstrated that twelve species of fungi belonging to four genera were isolated and identified. *Acremonium strictum* was the predominant species, succeeded by *Aspergillus niger*, *A. flavus*, *A. ustus*, and *A. terreus*. In addition to *Cladosporium cladosporioides*, three species of *Penicillium*—*P. aurantiogriseum*, *P. chrysogenum*, and *P. oxalicum*—were isolated and identified. There is a broad belief, further corroborated by this study, that the problems occurring at the surface of the thick juice are primarily attributed to fungi (Muir et al. 2019).

These microorganisms readily form a mat on the surface, containing species from genera such as *Aspergillus* and *Penicillium* (Bohn 1973, Willems et al. 2003). Ensuring food safety for customers is seen as a crucial global problem. Issues pertaining to various forms of contamination detrimental to human health have escalated in recent years.

The globalization and advancement of an exchange-oriented global economy have significantly impacted and expanded the food market. Simultaneously, the enhanced marketing of food products heightened the risk of exposure to both natural and manmade pollutants.

The existence of toxigenic species of fungi and their secondary metabolites is a significant concern in food safety. These fungi can produce numerous secondary metabolites, the functions of which remain mostly unidentified. Among these metabolites, mycotoxins, distinguished by their low molecular weight, may exhibit harmful effects on several physiological systems in humans and animals (Moretti and Susca 2017).

Aspergillus is one of the three most significant fungal genera in food spoilage and mycotoxin generation, alongside *Fusarium* and *Penicillium*. *Aspergillus* species exhibit optimal adaptation for development in tropical climates, since prevalent species infrequently flourish below 10 °C and mostly grow at temperatures of 37 °C or higher (Pitt and Hocking 2009).

The majority of organisms prevalent in food are xerophilic, with significant toxin producers capable of growing at or near 0.80 water activity (Pitt and Hocking 2009, Cabañes and Bragulat 2018).

The emergence of fungal species capable of producing hazardous compounds in the beet thick juice has garnered significant interest in food safety concerns. Mycotoxins, which are typically low molecular weight chemicals, result from the secondary metabolism of toxigenic fungi. They may pose a significant threat to human health globally, since they can cause a wide spectrum of dangerous biological activities (Taniwaki et al. 2018).

The microbial community of high-sugar food is predominantly composed of xerophilic filamentous fungi and osmophilic yeasts (Grant 2004). Numerous osmotolerant microorganisms, exhibit tolerance to elevated salt concentrations (Scott 1957, Grant 2004). However, the converse is not invariably applicable; microorganisms isolated from saturated salt lakes ($a_w = 0.75$), for instance, demonstrated an inability to withstand analogous a_w values induced by organic solutes (Kushner 1978, Cray et al. 2013). A wide variety of microbes, such as yeasts, and filamentous fungi, choose the environment with less water activity habitat. Species of yeasts and filamentous fungi are the only organisms that can thrive in environments with high sugar levels.

In addition, Sarkar et al. (2022) found that conditions with water activity less than 0.85 had a much higher diversity of fungi compared to all other groups combined. Previous research on the storage of beet thick juice has identified various fungal species, including *Candida bombi*, *C. zemplinina*, *Cordyceps sinensis*, *Cystofilobasidium infirmo-miniatum*, *Debaryomyces hansenii*, *Galactomyces geotrichum*, *Penicillium camemberti*, *Pichia* spp., *Pichia anomala*, *Rhodotorula minuta*, *Torulaspora* spp., and *Torulaspora delbrueckii*, all of which are recognised to inhabit food matrices such as cheese, yoghurt, wine, maple sap and sugar molasses (Sheneman and Costilow 1959, Samaraweera et al. 1995, Caggia et al. 2001, Petersen et al. 2002, Passoth et al. 2006).

The current results revealed the isolation and identification of *Acremonium*, *Aspergillus*, *Cladosporium*, and *Penicillium*. In tropical regions, like Egypt, the warm and humid climate provides ideal conditions for their growth. In tropical regions, *Aspergillus* and *Penicillium* play dual roles as beneficial microbes and serious spoilage agents. Effective management of their presence in thick juice storage systems, is essential to reduce their negative impacts. To mitigate these risks, strict hygienic protocols must be followed during juice concentration and storage. Additionally, airtight, temperature-controlled tanks with consistent monitoring of microbial indicators are recommended to inhibit fungal growth and preserve juice quality over time.

The long-term storage of beet thick juice, especially under inadequate sanitary or temperature conditions, may result in microbial contamination, with fungi being prominent spoiling agents.

These microbes can endure high-sugar conditions owing to their osmophilic characteristics, resulting in fermentation, discoloration, and the development of off-flavors. Moreover, fungal metabolism may release organic acids and mycotoxins, so further diminishing the physicochemical stability and safety of the stored juice.

The presence of fungi influences crystallization efficiency by modifying juice viscosity and pH, which could reduce sugar yield and purity. To preserve the quality of thick juice during prolonged storage, it is imperative to follow strict sterile measures, guarantee airtight storage conditions, and control temperature and humidity to prevent microbiological proliferation (Willems et al. 2003, Justé et al. 2008a, Justé et al. 2008b). This involves cleaning and sanitizing the storage tanks in order to decrease the initial microbial load (Gooddard 1997) and/or incorporating preservatives (biocides) to regulate the growth of existing microorganisms in the thick juice (Pollach et al. 1999), in addition to coating the surface of the thick juice with for example a 25 % sodium hydroxide solution to prevent the development of a microbial mat (van der Poel et al. 1998, Hein et al. 2002).

This type of degradation is regarded by certain companies as an important aspect of juice storage expenses, while others frequently view it as a justification for implementing preventative measures during storage. The findings of this investigation indicated that a temperature of 15 °C was optimal for beet thick juice, as it resulted in the lowest fungal CFUs, whereas 35 °C exhibited the highest overall fungal count. At all three storage temperatures—15, 25, and 35 °C—the control tanks exhibited higher CFUs compared to the treated tanks.

The CFUs in the control tanks exhibited a direct proportional increase with temperature, with 35 °C yielding the highest CFUs in comparison to 15 and 25 °C. Tanks containing the biocide hop β -acid demonstrated the most significant antifungal activity at temperatures of 15, 25, and 35 °C, as indicated by the lowest fungal growth recorded at 45, 130, and 174 CFUs, respectively.

The literature presents an evaluation of various biocides for their inhibitory effects on microbial growth, primarily focusing on yeasts and fungi in both the bulk and surface growth of stored beet thick juice.

The biocides assessed for controlling bulk contamination comprised thiocarbamate Busan 881/981, silver peroxide Huwasan, and hop β -acids, all at a concentration of 100 ppm active substance. An aqueous solution containing 25% sodium hydroxide (NaOH) was evaluated for its efficacy in inhibiting surface growth of fungi. Formaldehyde was evaluated for its role as a positive control in the experiments, owing to its proven effectiveness in microbial control, facilitating comparisons with alternative preservatives. Moreover, since hop β -acids demonstrate no activity against yeasts and fungi (Pollach et al., 1999), these results align with our findings.

Conclusions

This study constructed pilot plant consisting of twelve storage cylinders for the storage of thick beet juice at temperatures of 15, 25, and 35 °C. Hop β -acids and KEOCID 310 biocides were applied alongside surface sealing using 25.0 % NaOH with air removal. Twelve species of fungi related to four genera were identified.

These were *Acremonium strictum*, *Aspergillus brasiliensis*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. terreus*, *A. ustus*, *A. versicolor*, *Cladosporium cladosporioides*, *Penicillium aurantiogriseum*, *P. chrysogenum*, and *P. oxalicum*. The findings demonstrated that 15 °C represented the optimal storing temperature, resulting in the lowest fungal count, while 35 °C showed the highest overall fungal count.

The CFUs in the control tanks showed a direct proportional increase with temperature, with 35 °C resulting in the highest CFUs compared to 15 and 25 °C.

Tanks with hop β -acid exhibited increased antifungal activity at temperatures of 15, 25, and 35 °C, as indicated by the lowest fungal count. This study provides insights into optimal conditions for cost-effective storage in high-temperature regions, which could be advantageous for designers in the sugar industry.

Recommendations

Future studies should explore the efficacy of biocide mixtures or natural antifungal agents under industrial storage conditions. Additionally, assessing the scalability of fungal control strategies and evaluating microbial behavior over extended storage durations would provide deeper insight into long-term quality preservation of thick juice. Also, investigating the behavior of microbial communities under extreme heat (≥ 40 °C) would be of interest, particularly in the context of climate resilience and storage risk management in hot regions like Egypt.

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Author contributions

All authors contributed equally to data analysis, writing, and revising the article. All authors have reviewed and approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

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