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Research Paper / Araştırma Makalesi

Comparative Bioaroma Profiles from Sugar Beet Molasses Using *Saccharomyces cerevisiae* and *Saccharomyces boulardii* Under Extended Fermentation

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ABSTRACT

Flavor is a crucial factor that influences the food preferences of many consumers, and in recent years, there has been a growing consumer demand for the use of natural and ecologically sustainable flavor compounds in foods. This study investigated the volatile compound production abilities of *Saccharomyces cerevisiae* TGM10 and *Saccharomyces cerevisiae* var. *boulardii* S11 yeasts in sugar beet molasses, a significant by-product of the sugar industry under extended fermentation. The effect of fermentation time on the volatile compound profile was also determined. In fermentation carried out in Erlenmeyer flasks for up to 120 hours, both strains tolerated the medium containing 10% sugar beet molasses well. The volatile compound profiles of samples fermented by each strain for 96 and 120 hours were determined by GC-MS. *S. cerevisiae* TGM10 yielded a higher-alcohol–dominated profile, whereas *S. boulardii* S11 produced an ester-dominant profile. Results showed that both strains were capable of producing a variety of pleasant and valuable aroma compounds through the fermentation of sugar beet molasses. Additionally, yeast strain used in fermentation and fermentation time had significant effects on volatile compound profiles. In conclusion, *S. cerevisiae* TGM10 and *S. boulardii* S11 showed strong potential for low-cost production of bioaroma additives and might enhance flavor when employed as starter or adjunct cultures in fermented foods.

Keywords: *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, Fermentation, Bioaroma, Waste management

Şeker Pancarı Melasından *Saccharomyces cerevisiae* ve *Saccharomyces boulardii* Kullanılarak Uzatılmış Fermantasyon Süreci İle Elde Edilen Karşılaştırmalı Biyoaroma Profilleri

ÖZ

Lezzet, pek çok tüketicinin gıda tercihlerinin şekillenmesinde rol oynayan önemli bir faktör olup, son yıllarda gıdalarda doğal ve ekolojik olarak sürdürülebilir lezzet bileşiklerinin kullanılmasına yönelik artan bir tüketici talebi bulunmaktadır. Bu doğrultuda gerçekleştirilen bu çalışmada, *Saccharomyces cerevisiae* TGM10 ve *Saccharomyces cerevisiae* var. *boulardii* S11 mayalarının şeker endüstrisinin önemli bir atığı olan şeker pancarı melası ortamında uçucu bileşik üretme yetenekleri araştırılmıştır. Ayrıca, fermantasyon süresinin uçucu bileşik profili üzerindeki etkisi de belirlenmiştir. Erlenmeyer şişelerde 120 saate kadar gerçekleştirilen fermantasyonlarda her iki suş da %10 şeker pancarı melası içeren ortamı iyi bir şekilde tolere etmiştir. Her bir suş tarafından 96 ve 120 saat boyunca fermante edilen örneklerin uçucu bileşik profilleri GC-MS ile belirlenmiştir. *S. cerevisiae* TGM10 daha yüksek alkollerle baskın bir profil oluştururken, *S. boulardii* S11 esterlerin baskın olduğu bir profil üretmiştir. Sonuçlar, her iki suşun da şeker pancarı

melasının fermantasyonu yoluyla çeşitli hoş ve değerli aroma bileşikleri üretebildiğini göstermiştir. Ayrıca, fermantasyonda kullanılan maya suşunun ve fermantasyon süresinin uçucu bileşik profilleri üzerinde önemli etkileri olduğu tespit edilmiştir. Sonuç olarak, *S. cerevisiae* TGM10 ve *S. boulardii* S11'in, düşük maliyetli biyoaroma katkı maddesi üretimi için güçlü bir potansiyel gösterdiği ve fermante gıdalarda başlatıcı veya yardımcı kültür olarak kullanıldıklarında aromayı iyileştirici olarak rol oynayabilecekleri değerlendirilmiştir.

Anahtar Kelimeler: *Saccharomyces cerevisiae*, *Saccharomyces boulardii*, Fermantasyon, Biyoaroma, Atık yönetimi

INTRODUCTION

Flavor (taste and aroma) has a very important role in consumers' food preferences [1], and various flavoring compounds may need to be added to foods as additives to increase the preference for some foods. Aroma compounds are among the most used additives in the food industry [2]. These compounds are conventionally obtained through chemical synthesis (synthetic) or extraction (natural) from plant and, rarely, animal sources [3]. Although it is possible to produce aroma compounds at low cost and in high yields through chemical synthesis, the production of synthetic aroma compounds can cause ecological problems. In recent years, there has been a prejudice among consumers against synthetic products [4]. However, the production of aroma compounds from plant sources involves a laborious and expensive process [5]. Increasing consumer demand for natural foods and food ingredients has led to research into alternative, inexpensive, and sustainable new ways to produce flavor compounds. In this context, production through fermentation has significant potential, as it is selective, environmentally friendly, and sustainable [6]. Since natural substrates are used in fermentation production, these products can be labeled as natural according to both European Union [7] and United States of America [8] legislations.

Yeasts stand out due to some of their advantages in producing bioaromatic compounds by fermentation (such as their ability to produce a wide range of volatile compounds and to utilize agricultural wastes) [9]. In the production of bioaroma compounds from yeast through fermentation, various factors, including the strain used, fermentation time, properties of the substrate, temperature, pH, aeration, and agitation, have an effect [10].

Various agro-industrial wastes have a high potential for use in the production of aroma compounds, as they can provide environmentally friendly, low-cost, and sustainable production, while also creating an ideal culture medium for microorganisms involved in biocatalytic transformations [2]. In this context, molasses, a by-product of the sugar industry rich in sucrose, stands out due to both its high quantity and its chemical composition, and can cause significant pollution if not properly treated before being discharged into the environment [11].

Although there are many studies [12-14] in the literature examining the bioaroma production ability of *Saccharomyces cerevisiae*, research on *S. cerevisiae* var. *boulardii* is quite limited and has mostly focused on its probiotic and health-related functions rather than its capacity to produce aroma-active compounds. To the best of our knowledge, studies evaluating the volatile compound production of *S. boulardii* using food-grade substrates such as sugar beet molasses are scarce, and none have systematically investigated the effects of extended fermentation times on the volatile compound profile. In a previous study [15], we compared the volatile compound profiles of yeast extracts obtained from *S. cerevisiae* TGM10 and *S. boulardii* S11 via sugar beet molasses fermentation and observed that *S. boulardii* S11 exhibited high volatile compound production potential. Accordingly, this study aimed to determine the bioaroma compounds of ferments obtained as a result of extended (96 and 120 hours) sugar beet molasses fermentations of *S. cerevisiae* TGM10 and *S. boulardii* S11 strains and compare them. The hypothesis and experimental framework of the study are summarized in Figure 1.

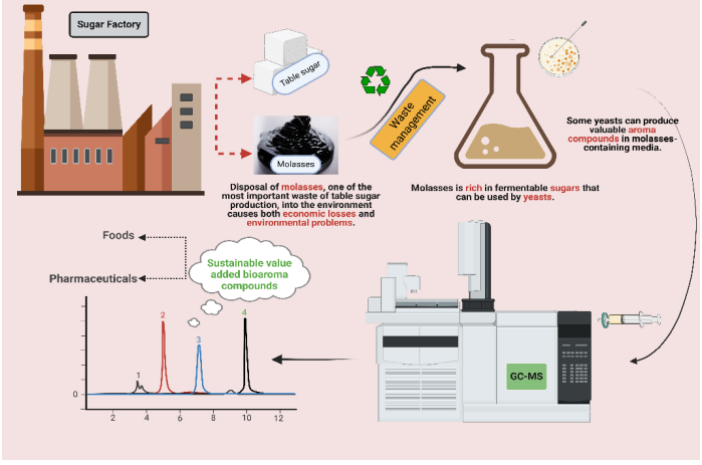


Figure 1. The hypothesis and experimental framework of the study

MATERIALS and METHODS

Microorganisms and Media

S. cerevisiae TGM10, and *S. cerevisiae* var. *boulardii* S11 were obtained from the microbial culture collection of Yildiz Technical University, Department of Food Engineering (Istanbul, Türkiye). Stock cultures were preserved in malt extract broth (LAB M, UK) with 25% sterile glycerol (Riedel-de Haën, Seelze, Germany) at -80°C.

Sugar beet molasses was supplied from Adapazarı Sugar Factory (Adapazari, Türkiye). According to the manufacturer's specifications, molasses contained 81.90% dry matter, 48.34% total sugars (including 47.29% sucrose and 1.05% invert sugar), and 12.22% ash. The pH of a 10% (w/v) solution, determined at 25°C after autoclaving, was 5.8. Molasses was kept sealed at 4°C until used. To precipitate heavy metals, molasses was diluted with distilled water at a 1:1 ratio and adjusted to pH 4.5-5.0 using 2 M H_2SO_4, followed by boiling. The mixture was then cooled to room temperature and centrifuged at 9000 rpm for 30 min using a Hitachi Centrifuge (Tokyo, Japan), and the resulting supernatant was used in the experiments [15].

Fermentation Experiments

For fermentation experiments, 200 g of 10% (w/w) sugar beet molasses medium was prepared with distilled water in 500 mL Erlenmeyer flasks and autoclaved at 121°C for 15 minutes. The yeast cultures were grown in malt extract broth at 30°C for 48 hours. After the incubation period, yeast counts were determined by the spread plate technique. Subsequently, 100 µL of each yeast culture (10^5-10^6 CFU/mL) was individually transferred to the fermentation medium. All fermentations were conducted in sealed flasks at 30°C with an agitation rate of 120 rpm for 96 and 120 hours. During fermentation, samples were taken from the medium every 24 hours under aseptic conditions, and yeast cell counts were made by the spread plate technique.

Determination of Volatile Compounds

Extraction and determination of volatile compounds of ferments were carried out by making some modifications to the methods proposed by Demirgül et al. [15] and Kıvançlı and Elmacı [16]. Volatile compounds in ferments were extracted by SPME (Solid Phase Microextraction) coupled with an AOC-5000 plus multifunctional auto-sampler (Shimadzu, Tokyo, Japan) attached with the Gas Chromatography-Mass Spectrometry (GCMS-QP2010, Shimadzu, Tokyo, Japan). 5 mL of the ferments taken from the 96 and 120 hours fermentation experiments under aseptic conditions were transferred to 15 mL vials and vortexed. DVB/CAR/PDMS (divinylbenzene/ carboxen/polydimethylsiloxane, 30/50 µm) SPME fiber (Supelco, USA) was used in the extraction process, and before extraction, the fiber was preconditioned at 250°C for 30 minutes, and then the samples in the vials were preheated at 60°C for 10 minutes. Extraction from ferments was carried out at 58°C for 55 minutes. After extraction, volatile compounds in the samples were separated using a Restek Rtx-5MS capillary column (30 m × 0.25 mm × 0.25 µm, USA) with helium as the carrier gas at a flow rate of 1.45 mL/min. The initial oven temperature was held at 40°C for 3 minutes, then increased to 100°C at a rate of 8°C/min, and then increased to 200°C at a rate of 5°C/min. The final temperature was increased to 250°C at a rate of 10°C/min and held at this temperature for 10 minutes. The injection temperature was set at 250°C. MS conditions were set to have an ionization energy of 70 eV, an interface temperature of 280°C, and a mass range of 50-400 amu. WILEY Registry of Mass Spectral Data and NIST (National Institute of Standards and Technology) libraries were used to identify volatile compounds in the samples. Molasses that was not inoculated with the culture was used as a control.

Statistical Analysis

Statistical evaluation was performed using SPSS (Version 23, USA). All experiments were carried out in triplicate, and the data were expressed as means±standard deviations (SD). A confidence level of 95% ($p \leq 0.05$) was used to determine statistical significance between values. In the analysis of volatile compounds, undetectable values were excluded, and appropriate statistical tests were applied depending on the number of groups for each compound. In volatile compounds and microbial count results with data for three or more groups, one-way analysis of variance (ANOVA) was applied to evaluate the differences between the groups, and Tukey test was used for post-hoc comparisons. For volatile compounds where data were available for only two groups, an independent sample t-test was applied. The cluster heatmap of the volatile compounds in the samples was formed using ClustVis (https://biit.cs.ut.ee/clustvis).

RESULTS and DISCUSSION

Monitoring Yeast Cell Numbers throughout Fermentation

The number of viable yeast cells at various fermentation times is presented in Table 1. After *S. cerevisiae* TGM10 and *S. boulardii* S11 strains were grown in malt extract broth for 48 hours, 100 µL each was inoculated into the fermentation medium, and the numbers of yeast strains in the counts made immediately after inoculation were 5.82 log CFU/mL and 5.12 log CFU/mL, respectively. The numbers of both strains decreased to a statistically insignificant ($p > 0.05$) level in the first 24 hours of fermentation (lag phase). At 48 hours, a significant increase ($p \leq 0.05$) was observed for both strains, after which cell numbers continued to increase slightly without statistical significance ($p > 0.05$) until the end of fermentation (120 hours). These results indicate that both strains tolerated the fermentation medium containing 10% sugar beet molasses well, with *S. boulardii* S11 showing comparatively higher viability. Similar to our results, Do et al. [17] also reported that the *S. cerevisiae* KCCM 51811 strain was able to tolerate a medium containing 10% molasses. In addition, the lack of

statistically significant differences in cell counts between 72, 96, and 120 hours suggests that fermentation had entered the stationary phase (Table 1). Volatile compound analyses were therefore focused on 96 and 120 hours in order to evaluate the impact of extended fermentation on volatile compound production.

Table 1. Number of *S. cerevisiae* TGM10 and *S. boulardii* S11 cells (log CFU/mL) at different fermentation times

Fermentation time (hour)	TGM10	S11
0	5.82 ± 0.10^{a}	5.12 ± 0.34^{a}
24	5.13 ± 0.14^{a}	5.04 ± 0.36^{a}
48	7.48 ± 0.44^{b}	7.40 ± 0.10^{b}
72	7.57 ± 0.43^{b}	7.66 ± 0.49^{b}
96	7.61 ± 0.31^{b}	7.93 ± 0.33^{b}
120	7.66 ± 0.69^{b}	8.04 ± 0.56^{b}

Values were expressed as mean value ± SD. Values marked with different letters in the same column are significantly different from each other ($p \leq 0.05$). CFU: colony forming unit.

Key Volatile Compounds

A total of 20 significant volatile compounds were identified in the ferments obtained by fermenting molasses with *S. cerevisiae* TGM10 and *S. boulardii* S11 strains for 96 and 120 hours: four alcohols, six aldehydes, nine esters, and one ketone. The relative abundances (%) of volatile compounds determined in ferments and unfermented molasses (control) are shown in Table 2.

Table 2. Effect of yeast strain and fermentation time on the key volatile compounds in sugar beet molasses fermentation

Volatile compounds	*S. cerevisiae* TGM10		*S. boulardii* S11		Control*
	96h	120h	96h	120h	
Alcohol					
2-Methyl-1-propanol	1.52** ± 0.22^{a}	1.57 ± 0.15^{a}	2.61 ± 0.36^{b}	2.99 ± 0.26^{b}	N.d.
3-Methyl-1-butanol	9.52 ± 2.00^{a}	10.32 ± 1.22^{a}	7.57 ± 1.17^{a}	8.26 ± 0.86^{a}	N.d.
2-Methyl-1-butanol	8.21 ± 0.21^{ab}	9.37 ± 1.37^{b}	6.32 ± 0.25^{a}	7.48 ± 0.48^{ab}	N.d.
2-Phenylethanol	8.22 ± 1.29^{a}	7.81 ± 0.35^{a}	2.67 ± 0.31^{b}	5.38 ± 0.33^{c}	N.d.
Aldehyde					
2-Methylbutanal	N.d.	N.d.	0.94 ± 0.09^{a}	1.91± 0.29^{b}	1.88 ± 0.28^{b}
3-Methylbutanal	N.d.	N.d.	1.82 ± 0.18^{a}	2.60 ± 0.13^{b}	1.96 ± 0.20^{a}
Phenylacetaldehyde	0.36 ± 0.16^{a}	N.d.	N.d.	N.d.	0.88 ± 0.14^{b}
Benzaldehyde	N.d.	N.d.	0.22 ± 0.09^{a}	N.d.	1.75 ± 0.23^{b}
Nonanal	1.20 ± 0.15^{a}	N.d.	2.86 ± 0.21^{b}	1.96 ± 0.32^{c}	1.34 ± 0.31^{ac}
Heptanal	N.d.	N.d.	0.98 ± 0.18^{a}	0.75 ± 0.13^{a}	N.d.
Ester					
Ethyl acetate	0.34 ± 0.10^{a}	1.65 ± 0.14^{b}	2.51 ± 0.35^{c}	1.08 ± 0.17^{d}	N.d.
Ethyl octanoate	N.d.	0.49 ± 0.24^{a}	5.61 ± 1.10^{b}	5.40 ± 0.48^{b}	N.d.
Ethyl decanoate	N.d.	0.36 ± 0.11^{a}	4.48 ± 0.70^{b}	3.74 ± 0.43^{b}	N.d.
Ethyl pentanoate	N.d.	N.d.	0.26 ± 0.12	N.d.	N.d.
Ethyl hexanoate	N.d.	N.d.	2.77 ± 0.38^{a}	3.44 ± 0.56^{a}	N.d.
Ethyl 9-decenoate	N.d.	N.d.	1.30 ± 0.42^{a}	0.63 ± 0.07^{a}	N.d.
Ethyl laurate	N.d.	N.d.	0.38 ± 0.06	N.d.	N.d.
Ethyl 9-hexadecenoate	N.d.	N.d.	1.53 ± 0.23^{a}	1.47 ± 0.21^{a}	N.d.
Ethyl 7-octenoate	N.d.	N.d.	0.94 ± 0.12^{a}	0.90 ± 0.20^{a}	N.d.
Ketone					
Acetoin	N.d.	N.d.	1.09 ± 0.19^{a}	2.60 ± 0.30^{b}	N.d.

Values were expressed as mean value ± SD. Values marked with different letters in the same row are significantly different from each other ($p \leq 0.05$). *Control sample was collected at t = 0, stored at 4 °C, and analyzed alongside the 96h and 120h samples; identical values were obtained. **Relative abundances expressed as % of total peak area. N.d.: Not detected.

2-Methyl-1-propanol is a higher alcohol that adds fruity flavor to foods such as beer [18], and this compound was found between 1.52% (*S. cerevisiae* TGM10, 96h) and 2.99% (*S. boulardii* S11, 120h) in all samples except the control. With this, *S. boulardii* S11 was statistically significantly ($p \leq 0.05$) more successful in producing this alcohol than *S. cerevisiae* TGM10. The change of this compound with time in fermentations carried out by the two yeast strains was insignificant ($p > 0.05$). 3-Methyl-1-butanol is a higher alcohol that is associated with different aroma notes (such as fruity and pungent) depending on the matrix of the food in which it is found and its concentration [19]. 2-Methyl-1-butanol is also a higher alcohol that can impart various aroma notes (such as whiskey, wine, fruity, and roasted) depending on the food matrix in which it is found [20]. In fermentations over the same period, both strains produced similar ($p > 0.05$) relative abundances of 3-methyl-1-butanol and 2-methyl-1-butanol. In addition, the relative abundances of 3-methyl-1-butanol and 2-methyl-1-butanol produced by both yeasts showed a statistically insignificant ($p > 0.05$) increase in the 120-hour fermentation compared to the 96-hour fermentation. Characterized by its rose-like floral aroma notes, 2-phenylethanol can contribute significantly

to the aroma of the food it is found in [21]. *S. cerevisiae* TGM10 strain was more successful in terms of 2-phenylethanol production (p≤0.05). In our previous study [15] comparing the volatile compounds of yeast extracts, we determined that *S. cerevisiae* TGM10 extract was richer in 2-phenylethanol compared to *S. boulardii* S11 extract. Interestingly, in the present study, the relative abundance of 2-phenylethanol decreased slightly (p>0.05) in the 120-hour fermentation obtained using the *S. cerevisiae* TGM10 strain compared to the 96-hour fermentation, whereas in fermentations obtained using *S. boulardii* S11, extending the fermentation to 120 hours increased (p≤0.05). Similar to our results, Patelski et al. [22] reported that *S. boulardii* CNCM I-745 and Alves et al. [23] reported that *S. cerevisiae* strains (CSc1 and CSc2) can produce higher alcohols, such as 3-methyl-1-butanol, 2-methyl-1-butanol, 2-methyl-1-propanol, and 2-phenylethanol.

The presence of aldehydes 2-methylbutanal (chocolate-like aroma) and 3-methylbutanal (dark chocolate-like aroma) [24] in the control sample suggests that these compounds were formed by Maillard reactions as a result of heat treatment [25]. The reason why these compounds were not detected in samples fermented by *S. cerevisiae* TGM10 may be the reduction of these aldehydes to higher alcohols (such as 3-methyl-1-butanol and 2-methyl-1-butanol) [26]. Indeed, the reason why these higher alcohols were higher in *S. cerevisiae* TGM10 samples compared to *S. boulardii* S11 samples suggests that this transformation is more effective in the *S. cerevisiae* TGM10 strain. With this, the relative abundance of these compounds decreased in the 96-h fermentation by *S. boulardii* S11 compared to the control. It increased in the 120-h fermentation, indicating that this strain can also synthesize these compounds. Phenylacetaldehyde, characterized by floral and fruity aromas [27], was present only in the 96-h sample (0.36%) fermented with *S. cerevisiae* TGM10 strain. However, this aldehyde was present in higher relative abundance (0.88%) in the control sample. The reason this compound could not be detected in the 120-h sample fermented with the *S. cerevisiae* TGM10 strain and in the samples fermented by *S. boulardii* S11 may be that it is reduced during the fermentation process and converted into a higher alcohol, such as 2-phenylethanol [26]. Benzaldehyde, which is generally characterized by an almond aroma [28], was present in lower relative abundance in the 96-h fermentation performed with *S. boulardii* S11 strain alone (0.22%) and compared to the control sample (1.75%) (p≤0.05). Here, similar to other aldehydes, it can be inferred that benzaldehyde may have been reduced to an alcohol, such as benzyl alcohol. However, the fact that this alcohol could not be detected in the samples suggests that the transformation may have occurred in a different manner, such as esterification [29]. Nonanal (citrus-like) and heptanal (oily and rancid) are products of lipid oxidation [30]. Nonanal was present in lower amounts (1.20%) in the 96-h sample fermented with *S. cerevisiae* TGM10 strain compared to the control (1.34%) (p>0.05), while it was not detected in the 120-h sample. In samples fermented with *S. boulardii* S11, although the nonanal level decreased statistically significantly (p≤0.05) as fermentation time increased, it remained higher than in the control sample. It is thought that this difference between yeast strains may be due to differences in lipid metabolism. Heptanal was only present in samples fermented with *S. boulardii* S11 strain and showed a statistically insignificant (p>0.05) tendency to decrease with increasing fermentation time. This suggests that this aldehyde may have decomposed or transformed into other compounds during fermentation. All these results necessitate more comprehensive studies to elucidate the ability of yeast cells to produce and metabolize aldehyde.

Both yeast strains produced ethyl acetate, which is associated with solvent-like aroma notes [31], at levels ranging from 0.34% (*S. cerevisiae* TGM10, 96h) to 2.51% (*S. boulardii* S11, 96h). However, as fermentation time increased, there was a significant (p≤0.05) increase in samples fermented by *S. cerevisiae* TGM10, while there was a significant (p≤0.05) decrease in samples fermented by *S. boulardii* S11. Ethyl acetate is frequently reported among the major volatile compounds produced during fermentations by various strains of *S. cerevisiae* [32, 33]. *S. boulardii* S11 was more successful than *S. cerevisiae* TGM10 in the synthesis of ethyl octanoate, characterized by sour apple-like aroma notes, and ethyl decanoate, characterized by floral odor notes [31]. Both esters were found only in the 120-h samples fermented by *S. cerevisiae* TGM10, while they decreased insignificantly (p>0.05) in the 120-h samples fermented by *S. boulardii* S11 compared to the 96-h sample. These results are consistent with previous findings by Saerens et al. [31], who reported that *S. cerevisiae* produces ethyl esters such as ethyl octanoate and ethyl decanoate during fermentation. Ethyl pentanoate (apple and fruit-like) and ethyl laurate (jujube and fruit-like) [34] esters were only present in *S. boulardii* S11 (96h) samples at 0.26% and 0.38%, respectively. Additionally, ethyl hexanoate (anise seed and apple-like aroma) [31], ethyl 9-decenoate (fruity aroma) [35], ethyl 9-hexadecenoate (fatty and floral aroma) [36], and ethyl 7-octenoate (fruity aroma) [37] esters were only found in samples fermented by *S. boulardii* S11. In samples fermented by *S. boulardii* S11, all esters except ethyl hexanoate decreased with increasing fermentation time, and this is thought to be related to the activity of esterase enzymes [31].

Acetoin, a ketone characterized by a yoghurt cream odor and a fatty buttery taste [38], was present only in samples fermented by *S. boulardii* S11 at levels between 1.09% (96h) and 2.60% (120h) (p≤0.05). Romano and Suzzi [39] examined 100 strains of *S. cerevisiae* for their acetoin production capacity, both in synthetic media and in grape must, and reported that acetoin production was generally low among the strains examined, although some strains showed higher production abilities. The results obtained in our study indicate metabolic differences between *S. boulardii* S11 and *S. cerevisiae* TGM10.

The heatmap generated based on these data to cluster the differences between volatile compounds in fermented and unfermented (control) samples is shown in Figure 2.

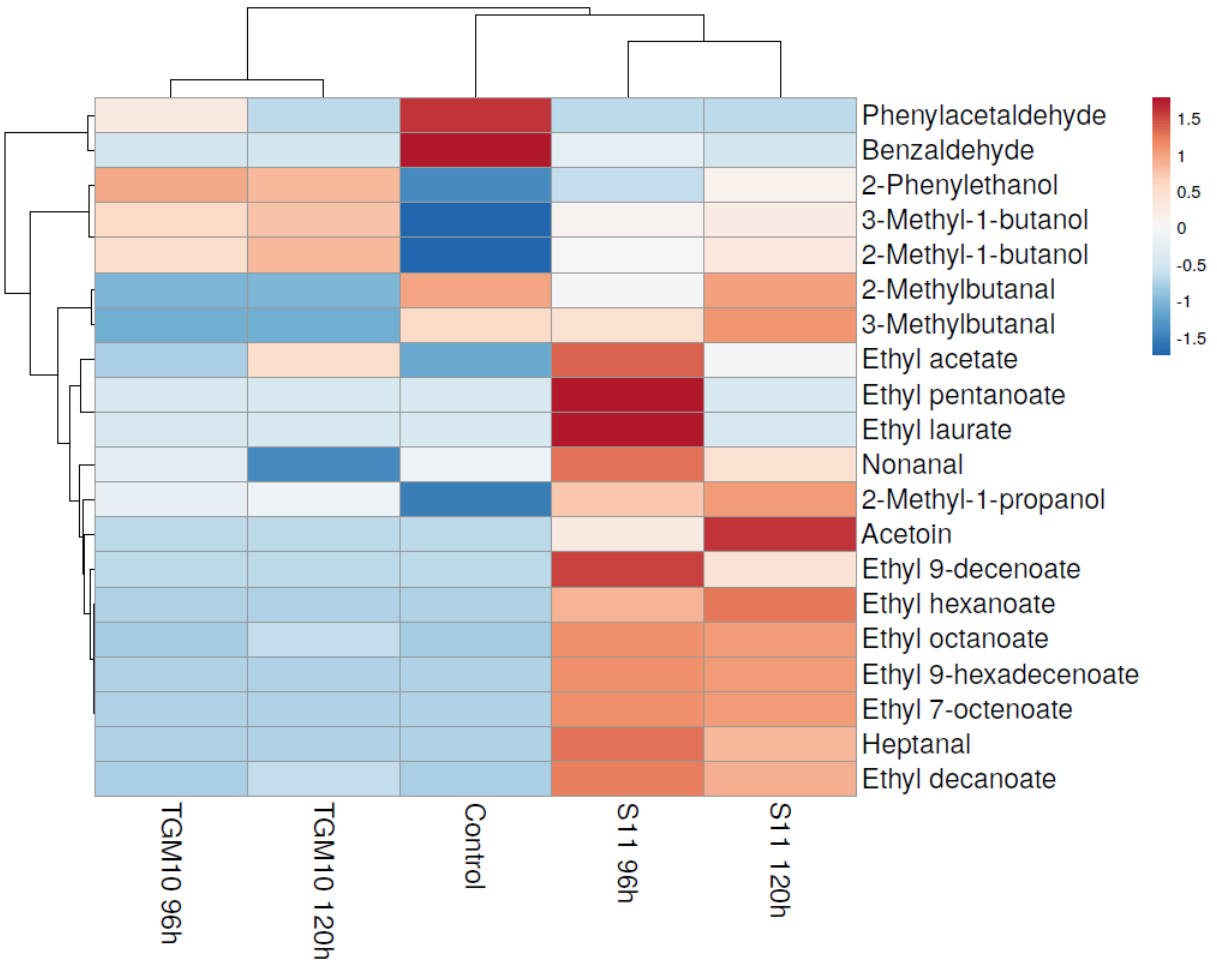


Figure 2. Heatmap visualization of cluster analysis of key volatile compounds determined in ferments obtained by *S. cerevisiae* TGM10 and *S. boulardii* S11 strains fermenting sugar beet molasses for 96 and 120 hours, and in unfermented molasses used as a control. The color code indicates relative abundance, ranging from red hues (high abundance) to white (medium abundance) and blue hues (low abundance).

As can be seen from Figure 2, *S. boulardii* S11 strain (especially in 96h fermentation) stands out in terms of its ability to produce esters and acetoin in general, while *S. cerevisiae* TGM10 strain stands out in terms of producing higher alcohols, although it is an exception. Although it can be said that both strains were unsuccessful in terms of aldehyde production, *S. boulardii* S11 strain stood out especially in terms of producing nonanal and heptanal (Figure 2). Extended fermentation is thought to cause the reduction of aldehydes to higher alcohols. Indeed, although there were exceptions, the relative abundance of higher alcohols increased with the extension of fermentation time. These results indicate that *S. boulardii*, although a variety of *S. cerevisiae*, has significant differences in terms of its ability to produce volatile compounds, and that fermentation time generally affects the volatile compound profile, both in terms of variety and proportion (Figure 2).

CONCLUSION

In this study, in which volatile compounds produced by *S. cerevisiae* TGM10 and *S. boulardii* S11 strains during prolonged fermentation of sugar beet molasses were evaluated, it was determined that both yeast strains could produce a generally pleasant and valuable aroma compound, such as 2-phenylethanol, in a cheap substrate such as molasses. With this, the yeast strain used in fermentation and fermentation time had significant effects on volatile compounds. For example, *S. boulardii* S11 was more successful than *S. cerevisiae* TGM10 in terms of the production of aroma metabolites such as various esters. This indicates the differences in metabolic properties of yeast strains and shows the importance of selecting the strain and fermentation time according to the targeted aroma profile. In addition, if probiotic *S. boulardii* is used in the production of flavoring or enhancing ferments, there is also the potential to release various postbiotic compounds beneficial to health into the environment. Therefore, elucidation of the postbiotic contents of samples fermented by *S. boulardii* S11 may encourage further use of this yeast in food fermentations.

CONFLICT of INTEREST

No potential conflict of interest was declared by the authors.

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