

Production of Some Higher Alcohols and Acetate Esters from Rice Bran by Yeasts Metabolisms of *Kluyveromyces Marxianus* and *Debaryomyces Hansenii*

Onur Güneşer

Uşak Üniversitesi: Usak Üniversitesi <https://orcid.org/0000-0002-3927-4469>

Y. Karagül Yuceer (✉ yoncayuceer@comu.edu.tr)

ÇOMÜ: Çanakkale Onsekiz Mart Üniversitesi <https://orcid.org/0000-0002-9028-2923>

Müge İşleten Hoşoğlu

Gebze Institute of Technology: Gebze Teknik Üniversitesi <https://orcid.org/0000-0001-8171-3018>

Sine Özmen Toğay

Bursa Uludağ Üniversitesi: Bursa Uludag Üniversitesi <https://orcid.org/0000-0002-8851-1803>

Murat Elibol

Ege University: Ege Üniversitesi <https://orcid.org/0000-0002-6756-6290>

Research Article

Keywords: Bioflavor, microbial fermentation, yeast metabolism, Gas chromatography olfactometry, sensory analysis

Posted Date: February 16th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-198090/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

The aim of this study was to evaluate the biosynthesis of flavor compounds from rice bran by yeast metabolisms. The microbial growth and flavor biosynthesis of *Kluyveromyces marxianus* and *Debaryomyces hansenii* in rice bran by microbial fermentation were investigated. Growth of both yeasts was assessed by the calculation of specific growth rates and doubling time. Their aroma biosynthesis was evaluated by gas chromatography-olfactometry (GC-O), gas chromatography-mass spectrometry (GC-MS) and *Spectrum™* sensory analysis. The specific growth rate (μ_{max}) and doubling time (t_d) of *K. marxianus* were calculated as 0.16/h and 4.21 h respectively, *D. hansenii* had 0.13/h of μ_{max} and 5.33h of t_d . *K. marxianus* and *D. hansenii* significantly produced higher alcohols and acetate esters from rice bran at high levels. Results showed that *K. marxianus* can produce 827.27 µg/kg of isoamyl alcohol, 169.77 µg/kg of phenyl ethyl alcohol and 216.08 µg/kg phenyl ethyl acetate during 24 h batch fermentation. Isovaleric acid was also synthesised by *K. marxianus* at high level (4013 µg/kg) in the batch fermentation of 96 hours. The highest concentration of isoamyl alcohol and phenyl ethyl acetate was determined as 415.64 µg/kg and 135.77 µg/kg, respectively at 24 h fermentation of *D. hansenii*. Fermented cereals and rose were defined as characteristic flavor descriptors for the fermented rice bran samples. Rose flavor term in fermented rice bran samples were found to associate with phenyl ethyl alcohol, phenyl ethyl acetate, isoamyl acetate and guaiacol. The valorization of rice bran can be achieved with the production of natural flavor compounds by yeast metabolisms.

Statement Of Novelty

Rice bran is commonly used for the rice bran oil, which is used in food feed, consumer care products. However, Rice bran is very sensitive to hydrolytic and oxidative rancidity to its rich oil content. For this reason, processing of rice bran has high cost to produce bioactive compounds. However, microbial fermentation had high efficiency and eco-friendly process. Valorization of rice bran to produce flavor compounds by yeast fermentation are considered first time with this study. Results showed that higher alcohols and acetate ester can be produced from rice bran with a high yield by the yeast metabolism

Introduction

Flavor compounds in foods either are formed naturally by the results of several chemical and biochemical reactions or are added as food additives during the manufacturing stages of foods. They are composed of several chemical compounds including acids, alcohols, aldehydes, esters, ketones, lactones, sulfur compounds, terpenes, and their derivatives. Over 6500 flavor compounds are identified and well characterized in food. But, about 300 of them are widely used as food additives [1–2]. While most of flavor compounds were produced synthetically by certain chemical reactions, a few of them are obtained from natural plant and animal sources such as mints, vanilla pods and beeswax by using conventional extraction and distillation methods [3].

Microbial fermentation has been gaining flavor scientists and manufacturers attention for decades because of flavor compounds produced enzymatic and microbiological processes are defined as "natural flavors" (European Council Directive, Directive 88/388/EEC). The growing of perception and desire for natural products by consumer also drawn on the development biotechnological process for the natural bio-products [4]. Today, agricultural wastes or by-products are emphasized as favorable sources in terms of economic and environmental impact to obtain a high added value product by microbial fermentation. Most of them have rich content of carbohydrates, protein, oil and other nutrients that are usable for microbial metabolisms [5–6]. Thus, several researches on using agro wastes or by-products for microbial production of flavor compounds have been conducted [7–12].

The rice bran is one of the main by-products in the rice milling. Rice bran has about 12–22% oil, 11–17% protein, 6–14% fiber, 10–15% moisture, and 8–17% ash, and comprises 10% of raw rice grain. It is also rich in some minor compounds including phytosterols, tocopherols, tocotrienols, B vitamins, phosphorus, and potassium. It is stated that the composition of rice bran was changed depending on many factors including rice variety, pretreatments for milling, degree of milling. China is the largest producer with a production of 10 million tons per year [13–15]. Due to higher nutritional value of rice bran, it is widely discussed for producing value added products such as rice bran oil, protein hydrolysate, γ-oryzanol, α-amylase and dietary fiber. But, it has not been fully utilized and also the researches on the potential usage of rice bran as raw material in the biotechnological process to produce high value added bioproducts are scarce [9, 13, 16]. In this context, Kaur and Chakraborty [9] optimized the production of vanillin from culture medium contained 15% of rice bran and %0.005 ferulic acid by fermentation of *Pediococcus acidilactici*. It was found that 1.269 g/L of vanillin can be obtained within 24 h fermentation at pH 5.6, at 37°C under shaking conditions at 180 rpm. Zheng et al. [16] reported that the highest yield reached 2.2 g/L of vanillic acid can be produced from waste residue of rice bran oil by the fermentation of *Aspergillus niger* CGMCC0774 in a 25 L fermenter. Rice bran has promising potential to use for producing natural flavor compounds by biotechnological process. This study

aimed to determine flavor production capabilities of *Kluyveromyces marxianus* and *Debaryomyces hansenii* from rice bran. The flavor production behavior of these yeasts was assessed based on the state of art flavor chemistry and bioprocess technique.

Materials And Methods

Material

Rice bran used in the present was obtained from local rice mill factory in Canakkale. The rice bran was stored at -18°C. Strains of *Kluyveromyces marxianus* NRRL YB-155 and *Debaryomyces hansenii* NRRL YB-6373 were obtained from ARS Culture Collection (NRRL collection, Peoria, Illinois). Analytical and chromatographic grades of chemicals are used in the all analysis in this study (Merck, Darmstadt, Germany, and Sigma-Aldrich, St Louis, USA).

Preparation of rice bran solution

Solution of (10%) of rice bran was used for both shake flask and bioreactor scale fermentation. In brief, the rice bran was grinded by knife miller Restch GM 200 (Haan, Germany) for 15 min. Then, 10% of rice bran solution was prepared with distilled water. The solution was homogenized at 24.000 rpm by Ultraturrax (IKA-WERKE GmbH, Germany) [11].

Preparation of microbial cultures

Adaptation and preparation of cell suspensions with *K. marxianus* and *D. hansenii* for rice bran fermentation were followed by the method mentioned by Guneser et al. [11]. Yeast extract Peptone Dextrose (YEPD) medium and YEPD medium with 0.3M NaCl were used for the cultivation of *D. hansenii* and *K. marxianus*, respectively. The cell concentration of *K. marxianus* and *D. hansenii* in microbial solution was determined by using Thoma slide under light microscope [17].

Fermentation experiments

For shake flask fermentation, 100 mL of the rice bran solution was poured into 250 mL Schott bottle with non-vented cap and a conventional sterilization procedure (121°C for 15 min) was applied by using autoclave (Hirayama, Saitama, Japan). Afterward, the rice bran solutions were inoculated using ca. 10^{6-7} cell per mL of each microbial solution. The flasks were incubated at 30°C for 72 h in a shaking incubator (Sartorius-Certomat IS, Goettingen, Germany) at 120 rpm. The rice bran solution without microorganism was used as control group and applied the same preparation procedure of the samples inoculated with microorganism.

Batch fermentation was performed by using stirred tank bioreactor (STR) (Biostat A-plus®, Sartorius, Melsungen, Germany) with a 4 L working volume. The STR has two six-blade impellers, pH probe (Hamilton, Easyferm K8/325) and PT 100 temperature sensor. Based on the shake flask fermentation, the batch fermentation condition was set as 0.325 vvm of the aeration rate, 120 rpm of the agitation speed, and 30°C of fermentation temperature for 120 h. The inoculation rate of the microbial solution for batch fermentation was 10^{6-7} cell per mL rice bran solution. Both the shake flask and batch fermentation experiments were performed in duplicate.

The growth curves of *K. marxianus* and *D. hansenii*

Growths of *K. marxianus* and *D. hansenii* were followed during shake flask and batch fermentations for evaluating the microbial growth behavior of the strains in rice bran. Specific growth rate (μ_{max}) and doubling time (t_d) of both yeasts were calculated for batch fermentation. The rice bran sample was taken from shake flask and bioreactor intermittently (24 h) in aseptic conditions. Pour plate counting technique was used in this study. YEPD with 0.3M NaCl agar for *K. marxianus* and YEPD agar for *D. hansenii* were used. Plates were incubated at 30°C for 72 h.

Gas chromatography- olfactometry (GC-O) and Gas chromatography- mass spectrometry (GC-MS) Analysis

Extraction of volatile compounds from the fermented rice bran samples were achieved by using the solid-phase microextraction (SPME) method combined with GC-MS and GC-O analysis. For this purpose, about 3 g sample was weighed into a 40-mL vial, and 1 g NaCl and 10 μ L internal standard (a mixture of 2-methyl valeric acid and 2-methyl 3-heptanone) were added. The samples were vortexed (Biosan V-1, Riga, Latvia) for 1 min. The vials were maintained at 40°C in a water bath for 20 min to equilibrate the volatile compounds in the headspace of the vials. Afterward, SPME needle (2 cm–50/30 μ m Divinylbenzene/Carboxen/Polydimethylsiloxane stable flex, Bellafonte, USA) was inserted into each vial. The SPME fiber was exposed at a depth of 2 cm in the headspace of the vial for 20 min. The SPME needle was injected into GC-O (HP 6890 with FID detector, Agilent, USA) or GC-MS (HP 6890 and 7895C MS, Agilent, USA).

The GC-MS condition was established as follow: HP5 MS column (30-m \times 0.25-mm i.d. \times 0.25- μ m film thickness, J&W Scientific, Folsom, CA); oven program: initial temperature 40°C for 5 min., ramp: 40 to 230°C at 10°C/min for 20 min.; a carrier gas and flow: Helium, at 1.2 mL/min. capillary direct interface temperature: 280°C; ionization energy: 70 eV; mass range: 35 to 350 amu; scan rate: 4.45 scans/s.

The GC-O condition was established as follow: column: HP-5 (30 m length \cdot 0.32 mm i.d., 0.25 μ m df; J&W Scientific); Column effluent: split 1:1 between flame ionization detector (FID) and olfactory port using deactivated fused silica capillaries (90 cm length, 0.25 mm i.d.); oven temperature: initial temperature 40°C for 5 min; ramp: 40 to 230°C at 10°C/min for 20 min.; the FID and sniffing port temperatures: 250°C and 200°C, respectively.

Gas chromatography olfactometry procedure was achieved in duplicate by two experienced panelists by using the method of Guneser et al. [11]. The National Institute of Standards and Technology (NIST) and Wiley Registry of Mass Spectral Data bases were used for the identification of volatile compounds. Quantitative analysis of the volatile compounds determined by GC-MS was performed as described by Avsar et al. [18].

Sensory analysis

Spectrum™ method was used to determine the sensory properties and changes in aroma profiles of the rice bran samples. Sensory evaluation was conducted with seven trained panelists (4 female and 3 were male, aged from 24 to 45 years). Panelists quantified the attributes using 15-point product-specific scale anchored to the left with 'not' and to the right with 'very' [19].

Statistical analysis

Two-way analysis of variance analysis (ANOVA) was performed to evaluate the growth of both yeasts in rice bran during the shake flask fermentation. One-way ANOVA analysis was also performed to determine the differences in the intensities of flavor compounds in the rice bran samples during fermentation. Tukey's test was applied to compare statistically significant differences at a significance level of $p \leq 0.05$ [20]. For all statistical analyses, SPSS for Windows (version 15.0) packed program was used. Principal component analysis (PCA) was performed by using the XLSTAT statistical program (trial version, 2015, Addinsoft, Inc., New York, NY, USA) to evaluate the relationship between the intensities of produced flavor compounds and their sensory impacts or perceptions.

Results And Discussion

Growth behavior of *K. marxianus* and *D. hansenii* in rice bran

The changes in number of cells of *K. marxianus* and *D. hansenii* in rice bran during shake flask fermentation are presented in Fig. 1.

During shake flask fermentation, significant increases were observed in the number of the cells for both yeast ($p \leq 0.05$). The number of *K. marxianus* cells increased slightly during 48 h fermentation, and the number of *K. marxianus* reached to the level of 2.47 log CFU/mL at the end of the fermentation. Unlike *K. marxianus*, the cell numbers of *D. hansenii* steady increased from 7.0 log CFU/mL to 10.69 CFU/mL. In case of bioreactor fermentation, the same growth behavior was observed for both yeasts. The numbers of *K. marxianus* and *D. hansenii* were found to increase in the level of 2.30 log CFU/mL and 3.28 log CFU/mL, respectively during 120 h bioreactor fermentation. While the specific growth rate (μ_{max}) and doubling time (t_d) of *K. marxianus* were calculated as 0.16/h and 4.21 h, *D. hansenii* had 0.13/h of μ_{max} and 5.33h of t_d . When calculated values of μ_{max} and t_d were compared for both yeasts, it can be concluded that the growth of *K. marxianus* was faster than *D. hansenii* in the rice bran fermentation (Fig. 2).

The findings of the present study are in good agreement with the results of previous studies [11, 21–22]. Zafar and Owais [22] reported that the maximum growth rate of *K. marxianus* was 0.157/h in the production of ethanol by crude whey fermentation. In another study by Guneser et al. [11], the specific growth rates for *K. marxianus* and *D. hansenii* were calculated as 0.08/h and 0.177/h in the tomato pomace fermentation, respectively. Goshima et al. [23] reported higher growth rate for the strains of *K. marxianus* NBRC177 and DMB1 than those of the present study. The researchers determined that the growth rates varied between 0.8/h and 1.06/h depending on the fermentation temperature for these strains in the bioethanol production from lignocellulosic hydrolysates. Compared to our findings, lower growth rates for *K. marxianus* and *D. hansenii* were also reported by previous studies [24, 25]. In a study by Madawati et al. [24], the growth rate for *D. hansenii* was determined between 0.03/h and 0.06/h in arabitol production from by-products of *Reutalis trisperma* biodiesel. Similarly, Sharma et al. [25] reported 0.02/h of growth rate for *K. marxianus* NIRE-K3 (MTCC 5934) in the YEPX medium with xylose (3%) medium at 45°C. Overall, it can be concluded that variation in growth rates of *K. marxianus* and *D. hansenii* results from strain type, utilization behavior of substrate by the microbial strains, agro waste types and its physicochemical structure and fermentation conditions. In general, several pre-treatment processes such as acidic or enzymatic hydrolysis are addressed for promoting of microbial growth on agro waste. But, the cost of pre-treatment should be taken into careful consideration in fermentation processes [26, 27].

Flavor production characteristics of *K. marxianus* and *D. hansenii* in rice bran

In the present study, flavor production behaviors of *K. marxianus* and *D. hansenii* in rice bran were determined by using GC-O and GC-MS techniques. Aroma-active compounds, which produced by both yeasts, and their intensities were determined in the shake flask fermentation stage by GC-O analysis. Total 33 aroma-active compounds were identified in the fermented and unfermented rice bran samples. Identified aroma-active compounds composed of acids, alcohols, aldehydes, esters, ketones, and pyrazines (Table 1). As seen in Table 1, higher alcohols and acetate esters among the aroma-active compounds were the most abundant compounds in the fermented rice bran samples. The intensities of isovaleric acid (sour, dirty), isoamyl acetate (fermented fruit), phenyl ethyl alcohol (rose) and phenyl ethyl acetate (floral) in rice bran samples fermented by both yeasts were found to be higher than the unfermented samples. It was also found that the rice bran samples fermented by *K. marxianus* had higher intensities of guaiacol (cotton candy) and 2,4-nonenal (oxide oil) than others, whereas 2,5-dimethyl-3-ethylpyrazine (dirty), 2-heptanone (metallic), 2-heptanal (oxide), 1-octen-3-ol (mushroom) was perceived at the highest intensities in rice bran samples fermented by *D. hansenii*.

Table 1
Aroma active compounds of the fermented and unfermented of rice bran.

Ri ^a	Aroma active compound	Aroma Quality	Methods of identification	Aroma Intensity ^a (Mean±S.E)		
				Control	<i>K. marxianus</i>	<i>D. hansenii</i>
<500	Methanethiol	Sulphur	RI, Odor	0.5±0.30	0.9±0.07	1.3±0.20
584	Diacetyl	Diacetyl, butter	RI, MS, Odor	3.8±0.50	0.5±0.30	Nd.
658	Acetic acid	Vinegar	RI, Odor	0.5±0.30	1.0±0.70	1.5±0.30
789	Isobutyric acid	Bubble gum	RI, MS, Odor	Nd.	0.5±0.30	Nd.
799	Hexanal	Green, grass	RI, MS, Odor	2.3±1.62	0.5±0.30	2.0±0.70
804	Isobutyl acetate	Sweet,	RI, MS, Odor	0.8±0.50	Nd.	Nd.
861	Isovaleric acid	Sour, dirty	RI, MS, Odor	4.0±0.10	6.3±0.20	3.5±1.70
872	Unknown 1	Medicine	RI, Odor	2.5±1.76	0.8±0.50	2.0±1.70
873	Isoamyl acetate	Fruity	RI, MS, Odor	1.0±0.10	4.8±0.20	2.8±0.20
899	2-heptanone	Metallic	RI, MS, Odor	2.5±1.76	Nd.	3.0±1.40
908	Methional	Boiled potato	RI, Odor	1.0±0.07	2.5±1.76	2.5±1.70
927	2-acetyl-1-pyrroline	Popcorn	RI, Odor	6.3±0.80	2.0±1.41	0.5±0.30
961	2-heptanal	Oxide, peanut	RI, MS, Odor	Nd.	Nd.	3.0±2.0
972	Octenone	Metallic	RI, MS, Odor	Nd.	2.0±1.41	Nd.
978	1-octen-3-ol	Mushroom	RI, MS, Odor	7.8±0.50	4.0±0.70	5.5±0.30
985	3,5-Octadien-2-one	Geranium	RI, MS, Odor	2.5±1.76	2.5±1.70	1.0±0.70
1000	Hexyl acetate	Cologne, fresh	RI, MS, Odor	2.8±0.20	1.3±0.50	Nd.
1025	2-acetylthiazole	Popcorn	RI, Odor	4.5±0.30	1.0±0.20	1.0±0.70
1060	(E)-2 -octenol	Dirty, nutty	RI, Odor	2.5±0.30	1.3±0.50	2.0±0.70
1083	Guaiacol	sweet, smokey	RI, MS, Odor	1.5±1.49	2.8±2.0	1.5±1.49
1096	2,5-dimethyl-3-ethylpyrazine	Dirty, dust	RI, Odor	5.0±0.01	4.8±1.20	6.5±0.30
1098	Sotolon	Burn spicy	RI, Odor	Nd.	1.5±1.0	Nd.
1109	2-acetyl-2-thiazoline	Popcorn	RI, Odor	3.0±0.07	2.0±1.40	Nd.
1133	(E)-2-Nonenal	Hay	RI, MS, Odor	1.5±0.30	1.0±0.70	0.8±0.50
1149	Phenylethyl alcohol	Floral, rose	RI, MS, Odor	Nd.	3.5±0.30	1.5±0.30
1169	(E)-linalool oxide	Dry herb, tea	RI, MS, Odor	1.5±1.0	2.0±1.40	1.3±0.90
1221	2,4-nonadienal	Oxide oil	RI, MS, Odor	0.5±0.30	1.5±0.30	Nd.
1247	3-carvomenthenone	Fishy	RI, MS, Odor	1.0±0.07	0.8±0.50	Nd.
1264	Phenyl ethyl acetate	Floral	RI, MS, Odor	Nd.	3.5±1.40	1.0±0.07
1269	Geraniol	Floral, lactone	RI, MS, Odor	1.5±1.00	2.0±1.40	1.5±1.0
1329	(E,E)-2,4-decadienal	Fried fatty	RI, MS, Odor	2.5±1.75	2.5±1.76	1.5±1.0
1348	Unknown 2	Cereal, wet bulgur	RI, Odor	2.0±1.40	2.5±1.76	1.0±0.07
1378	Dodecanal	Floral	RI, MS, Odor	2±0.07	3.0±2.10	2.5±176

^aThe post-peak intensity of aroma-active compound at 72 h fermentation time. MS: mass spectrum identification. RI: Kovats retention index. Nd.: not detected SE: standard error.

Gas chromatography olfactometry expresses the relative intensities of flavor compounds and their odor impacts. Therefore, the findings GC-O should be reviewed with the results GC-MS that indicate the quantities of the flavor compounds in the aroma research [12] [28]. Based on the results of GC-MS analysis, it was observed that six flavor compounds including isoamyl alcohol, isovaleric acid, isoamyl acetate, phenyl ethyl alcohol, phenyl ethyl acetate and guaiacol were produced from rice bran by *K. marxianus* and *D. hansenii* at higher concentrations in 72 h shake flask fermentation (Table 2). The concentration of 2,4-nonenal and 2,5-dimethyl-3-ethylpyrazine determined in the fermented rice bran samples did not increase. These findings could be related to the odor thresholds and concentrations of these compounds [29, 30]. Basically, the odor threshold of aroma compounds was affected by their vapor pressures and molecular interactions of other substances which existed in the medium. The odor threshold value was determined by sniffing flavor compound and defined as the lowest concentration of flavor compound which is required for the recognition of its odor. The intensity of flavor compound was directly related with the odor threshold. Therefore, even if the changes in the concentrations of flavor compounds is low, the intensities of them could be perceived as high [31].

The concentrations of higher alcohols and acetate esters in the fermented rice bran samples significantly increased during the shake flask fermentation ($p \leq 0.05$) (Table 2). Rice bran fermented by *K. marxianus* had 522 µg/kg of isoamyl alcohol and 111 µg/kg of phenyl ethyl alcohol while the concentrations of isoamyl alcohol and phenyl ethyl alcohol were determined as 445 µg/kg, and 108 µg/kg in rice bran fermented by *D. hansenii* respectively, which were higher than those of unfermented rice bran samples (Table 2) Apart from the primary metabolites (as carbon dioxide, ethanol and glycerol), acids, higher alcohols and esters are produced as the main secondary metabolites in the yeast metabolism. These secondary metabolites, which are produced by different metabolic pathways of yeast, contributed the flavor profile of several fermented foods and beverages such as bread and wine [32, 33]. In yeast metabolism, the synthesis of higher alcohols including isoamyl alcohol and phenyl ethyl alcohol are achieved by Ehrlich pathway, which contains several biochemical reactions of branched chain, aromatic and sulfur-containing amino acids such as transamination, decarboxylation, oxidation, and reduction. In the Ehrlich pathway, L-phenylalanine and L-leucine are the main substrate for the biosynthesis for isoamyl alcohol and phenyl ethyl alcohol, respectively.

Phenyl ethyl alcohol can also be synthesized from carbohydrate via Shikimate pathway in yeast cells [34–36]. Most knowledge of the Ehrlich and Shikimate pathways was obtained from studies on the fermentation behavior of *Saccharomyces cerevisiae*, several transaminases, decarboxylase, dehydrogenase enzymes are responsible for these pathways in the metabolism of *S. cerevisiae*, but the secondary metabolites production pathways of *K. marxianus* and *D. hansenii* and their enzymes systems are still being investigated [36, 37].

Table 2
Concentration of flavor compounds in rice bran fermented by *K. marxianus* and *D. hansenii*

Flavor compounds	Aroma Quality	Concentration of flavor compounds ^a ± S.E (µg/kg rice bran solution)		
		Control	<i>K. marxianus</i>	<i>D. hansenii</i>
Isoamyl alcohol	Banana	48.50±31.46 ^B	522±140 ^A	445±19.58 ^A
Isovaleric acid	Sour, fruity	65.48±8.20 ^B	210.17±34.76 ^A	285.60±0.56 ^A
Isoamyl acetate	Fruity	Nd.	153.78±13.09 ^A	120.10±2.44 ^A
Guaiacol	Burnt sugar	0.31±0.12 ^B	7.77±2.10 ^A	7.85±0.37 ^A
Phenyl ethyl alcohol	Rose	3.54±2.50 ^B	111±13.88 ^A	108.44±13.46 ^A
Phenyl ethyl acetate	Honey, Floral	2.32±1.64 ^B	205.93±17.14 ^A	35.99±13.72 ^B

^{A-B} Means followed by different uppercase letters represent significant differences for the same flavor compound in each yeast fermentation ($p \leq 0.05$). ^aRelative abundance of flavor compound at 72 h fermentation time. SE: standard error. ND: not detected.

There are two types of ester can be produced by yeast metabolism. They are acetate esters and medium-chain fatty acid ethyl esters. When the examined our findings, *K. marxianus* and *D. hansenii* are seen to produce especially acetate esters including isoamyl acetate and phenyl ethyl acetate from rice bran (Table 2). The concentration of phenyl ethyl acetate (205.93 µg/kg) in rice bran fermented by *K. marxianus* was found remarkably higher than that of *D. hansenii* (35.99 µg/kg) ($p \leq 0.05$). However, no significant difference was

observed between isoamyl acetate production rates of both yeasts ($p \geq 0.05$). While acetate esters composed of the acid and alcohol groups, medium-chain fatty acid ethyl esters contain the alcohol and medium-chain fatty acids. Both esters can be produced through lipid and acetyl-CoA metabolism and an *acyl transferase* or *ester synthase* enzymes play important role in the formation of esters in yeast. The synthesis of acetate esters were catalyzed by the *alcohol acetyltransferases I* and *II* encoded by the genes ATF1 and ATF2 in yeast [32, 33]. Moreover, the concentration of acetyl-CoA and a fusel alcohol and the activities of enzymes affected the rate of acetate ester formation. It was emphasized that the expression levels of the ATF1 and ATF2 during the fermentation are the most important factor for the concentration levels of the acetate ester as well as the substrate concentration in the medium [32, 38]. Therefore, higher phenyl ethyl acetate concentration in rice bran fermented by *K. marxianus* could be attributed the higher ATF1 and ATF2 expression compared to *D. hansenii*.

Concentrations of isovaleric acid and guaiacol increased significantly in the fermented rice bran during shake flask fermentation ($p \geq 0.05$). The concentrations of isovaleric acids in the rice bran samples fermented by *K. marxianus* and *D. hansenii* were found as 210.17 and 285.60 µg/kg, respectively (Table 2). Determination of isovaleric acid at higher concentrations in fermented rice bran samples could be attributed to the usage of the leucine, isoleucine and valine found in rice bran protein by both yeasts and their aminotransferase enzymes activities. Similarly, the synthesis of higher alcohols, it was emphasized that α -keto acids such as isovaleric acid can be also synthesized in the first step of the Ehrlich pathway. In this step, specifically leucine, isoleucine and valine can be converted to α -keto acids and the *aminotransferase* enzymes catalyzed this reaction [39].

Guaiacol is defined as phenolic off flavor in fermented beverages such as wine. Phenolic volatiles can be synthesized from the phenolic acids of the hydroxycinnamic series (e.g. 4-hydroxycinnamic, *p*-coumaric and 4-hydroxy-2-methoxycinnamic or ferulic acid) by decarboxylation reactions in yeast metabolism [40, 41]. In the present study, guaiacol was identified as aroma-active compounds at lower intensity in the rice bran samples fermented by *D. hansenii* and unfermented rice samples by GC-O analysis (Table 1). However, it was determined by GC-MS in all rice bran samples ranged from 0.31–7.85 µg/kg (Table 2). This difference may be attributed to the fact that molecular interactions of guaiacol with other substances which are found in the medium. Sensitivities of both analysis techniques may also lead to this difference [42].

The findings of the present study are in good agreement with the results of several previous studies on the production of aroma compounds from different agro wastes by yeast metabolism [7, 21, 38, 43–47]. In a recent study by Kilmanoğlu et al. [43], it was reported that higher alcohols and acetate esters including isoamyl alcohol, phenyl ethyl alcohol, phenyl ethyl acetate and ethyl acetate could be produced from tomato pomace hydrolysate by the fermentation with *K. marxianus* similar to our findings. The researchers also expressed that heat-treated dilute acid pretreatment and cellulolytic enzyme hydrolysis led to increase in the amount of higher alcohols and esters by increasing the fermentable sugars in tomato pomace. Similarly, Martinez et al. [48] showed that higher alcohols and esters can be produced from the mixture of sugarcane bagasse with sugar beet molasses by solid state fermentation of *K. marxianus* ATCC 10022. According to this, the researchers expressed that the maximum cumulative volatile production was achieved at 40°C, 35% molasses rate and specific air flow rate of 0.14 L/h g and the main volatile components composed of 43% alcohols and 18% ester species including ethanol, isoamyl alcohol, isobutyl alcohol, ethyl acetate, isoamyl acetate and isobutyl acetate. It was also observed that production of esters can be increased up to 35% with the working conditions as 30°C of fermentation temperature, 25% molasses rate, and specific air flow rate of 0.11 L/h g. Vong and Liu [44] investigated the flavor production capabilities of ten yeasts originated from wine and dairy foods in the soybean residues (okara) which is a by-product soy milk. The researchers expressed that soybean residues fermented by dairy yeasts including *Geotrichum candidum*, *Yarrowia lipolytica*, *Kluyveromyces lactis* and *D. hansenii* have musty and moldy aromas at higher intensities owing to the higher content of aldehydes and methyl ketones which are different from our findings about the fermentation of *D. hansenii*.

The changes in aroma compounds in the rice bran samples were also determined during batch fermentation in the present study. Table 3 shows the changes in the concentrations of aroma compounds in bioreactor conditions. It was observed that the concentration of the aroma compounds produced from rice bran changed significantly depends on the yeast types. In the *K. marxianus* fermentation, the synthesis of isoamyl alcohol and phenyl ethyl acetate was the highest at 24 h. Amount of both compounds significantly decreased from 48 h to 72 h of fermentation ($p \leq 0.05$). Hence, it was observed that the amount of phenyl ethyl alcohol increased from 5.42 µg/kg to 202.01 µg/kg during 96 h of fermentation. In the same manner, the synthesis of isovaleric acid and guaiacol was found to increase during 96h of fermentation. It was observed that the concentration of isoamyl acetate produced by *K. marxianus* increased during 48 h, then a sharp decrease in the concentration of this compound determined at the end of fermentation.

In case of fermentation by *D. hansenii*, isovaleric acid was not determined in the samples in bioreactor conditions. Like the fermentation by *K. marxianus*, the synthesis of isoamyl alcohol and phenyl ethyl acetate was the highest at 24 h and the concentration of these compounds were determined as 41.564 and 135.77 µg/kg, respectively. The highest phenyl ethyl alcohol concentration was determined at 48h. Moreover, it was observed that *D. hansenii* showed the similar synthesis behavior with *K. marxianus* in terms of guaiacol and isoamyl acetate production during bioreactor fermentation. However, *D. hansenii* produced lower amount of isoamyl alcohol than *K. marxianus* at the same fermentation time (Table 3). When the productivities of aroma compounds were examined during bioreactor fermentation, the highest productivity was calculated to produce isoamyl alcohol in both yeasts' fermentation followed by phenyl ethyl acetate and phenyl ethyl alcohol (Data not shown). Productivity of isoamyl alcohol for the fermentations of *K. marxianus* and *D. hansenii* were calculated as 34.46 µg/kg h and 17.31 µg/kg h. Hence, it was calculated that *K. marxianus* had a higher phenyl ethyl acetate productivity (9.00 µg/kg h) than that of *D. hansenii* (5.65 µg/kg h) while the productivity value of phenyl ethyl alcohol for both yeasts was calculated to be relatively close to each other.

Table 3
Changes in the concentration of flavor compounds produced from rice bran by *K. marxianus* and *D. hansenii* during bioreactor fermentation

Flavor compounds	Aroma Quality	<i>K. marxianus</i>					
		Concentration of Flavor Compounds ^a (µg/kg rice bran solution) Mean±S.E					
		Fermentation Time (hour)					
		0	24	48	72	96	120
Isoamyl alcohol	Banana	54.22±9.0 ^C	827.27±77.32 ^A	482.82±68.98 ^B	68.65±1.87 ^C	47.21±3.73 ^C	58.17±1.55 ^C
Isovaleric acid	Sour, fruity	200.±70.50 ^D	245.30±79.70 ^D	502±166 ^{CD}	2813±188 ^{AB}	4013±959 ^A	1073±283 ^{BC}
Isoamyl acetate	Fruity	0.94±0.21 ^C	36.27±1.63 ^B	59.23±5.36 ^A	0.49±0.06 ^C	0.47±0.07 ^C	Nd.
Guaiacol	Burnt sugar	0.23±0.04 ^C	11.66±2.58 ^C	36.57±2.52 ^{BC}	98.27±4.15 ^{AB}	134.32±28.03 ^A	66.88±5.20 ^{BC}
Phenyl ethyl alcohol	Rosy	5.42±1.10 ^B	169.77±42.22 ^A	163.17±11.34 ^A	118.73±10.67 ^{AB}	202.01±35.10 ^A	155.28±2.97 ^A
Phenyl ethyl acetate	Honey, Floral	2.56±0.48 ^B	216.08±31.67 ^A	46.09±1.80 ^B	2.18±0.10 ^B	1.96±0.43 ^B	0.4±0.03 ^B
Flavor compounds	Aroma Quality	<i>D. hansenii</i>					
		Concentration of Flavor Compounds ^a (µg/kg rice bran solution) Mean±S.E					
		Fermentation Time (hour)					
		0	24	48	72	96	120
Isoamyl alcohol	Banana	0.61±0.06 ^C	415.64±101.06 ^A	340.52±16.09 ^{AB}	75.89±4.12 ^C	99.77±37.35 ^{BC}	37.28±2.25 ^C
Isoamyl acetate	Fruity	0.16±0.04 ^B	46.18±10.53 ^A	34.16±1.22 ^A	0.79±0.54 ^B	0.20±0.08 ^B	0.28±0.02 ^B
Guaiacol	Burnt sugar	0.28±0.02 ^C	3.72±0.92 ^{AB}	3.18±0.06 ^{AB}	4.04±0.01 ^{AB}	5.98±0.79 ^A	2.18±0.08 ^{BC}
Phenyl ethyl alcohol	Rosy	3.09±0.10 ^C	106.52±25.54 ^{AB}	133.95±2.10 ^A	92.33±0.03 ^{AB}	127.08±25.79 ^A	37.94±3.47 ^{CD}
Phenyl ethyl acetate	Honey, Floral	0.05±0.04 ^B	135.77±36.64 ^A	30.26±1.87 ^B	1.04±0.01 ^B	0.58±0.10 ^B	0.26±0.05 ^B

^{A-D} Means followed by different uppercase letters represent significant differences for the same flavor compound during fermentation in each yeast fermentation ($p\leq 0.05$). ^aRelative abundance of flavor compound. SE: standard error. ND: not detected.

The findings of the present study are consistent with previous studies on the production of flavor compounds by fermentation [11, 12, 49–52]. Mederios et al. [51] reported increasing concentration of ethyl acetate until 22 h of fermentation time, and then ethyl acetate concentration significantly decreased in the solid state fermentation of cassava bagasse by *K. marxianus* on a packed bed column bioreactor. In a study by Guneser et al. [11], the highest production of isoamyl alcohol and phenyl ethyl alcohol in tomato pomace fermented by *K. marxianus* and *D. hansenii* was reported at 24 h fermentation and the concentrations of isoamyl alcohol and phenyl ethyl alcohol was determined in the ranges between 0.23-126.72 µg/kg and 3.30-158.72 µg/kg. Similarly, Yilmaztekin et al. [52] reported that the production of isoamyl alcohol from sugar beet molasses fermented by *Williopsis saturnus* var. *saturnus* in 5L bioreactor increased gradually during 288 h, while the production of isoamyl acetate gradually increased until about 140 h fermentation. In our previous study [12], we also observed similar production changes for mushroom-like flavors including 1-octen-3-ol, 1-octen-3-one and

octanol which were produced by fungal metabolism of *T. atroviride* and *A. sojae* in tomato and pepper pomaces in bioreactor fermentation.

After a certain time of fermentation, loss of aroma compounds produced in bioreactor condition can be related to many factors such as nutrients/precursor concentration, secondary metabolite inhibitions and aeration of bioreactor. Depending on these factors, several systems have been reviewed for recovery of aroma compounds from bioreactor system [1, 53, 54]. For instance, Tay [55] performed *in situ* product removal techniques for the production of isoamyl acetate from sugar beet molasses in bioreactor with batch and feed batch systems to avoid limiting factors. The researcher found that the maximum production of isoamyl acetate was 308.1 mg/L in the presence of macroporous resin H103 where only 38.4 mg/L of isoamyl acetate was produced without resin in batch fermentation. Unavoidably discharge of the aroma compounds from the bioreactor system with the exhaust gas defined as stripping which is considered the main reason for losses of aroma compounds during fermentation in the aerated bioreactors [49]. Urit et al. [49] modelled the stripping process for the production of ethyl acetate by *K. marxianus* on whey. The researchers expressed that the stripping of ethyl acetate highly depended on the aeration rate but was independent of the phase-transfer coefficient and the cooling the exhaust gas condenser of bioreactor system did not influence the stripping of the ethyl acetate.

Relationship between aroma profile and sensory characteristics of fermented rice bran

Total 13 descriptive flavor terms were defined in fermented and unfermented rice bran samples by the panelists and significant differences were observed between the rice bran samples in terms of boiled corn, sweet aromatic, fermented cereal, wet bran and rose aromas (Fig. 3). According to this, boiled corn was found to be the highest in the unfermented rice bran samples as control and no significant differences were observed between in rice bran samples fermented *K. marxianus* and *D. hansenii* regarding this aroma. In the same way, it was determined that both fermented rice bran samples had a higher fermented cereal flavor than control samples. Rose aroma in rice bran fermented by *K. marxianus* was determined noticeably higher than others.

For revealing of the relationship between sensory properties and flavor compounds in fermented rice bran solution, Principal Component Analysis (PCA) was performed based on the findings of sensory evaluation and GC-O analysis. It was determined that two principal components expressed by PCA the variations of sensory and aroma profiles of the rice bran samples. While the component PC1 was 59.53%, 40.47% of variations was expressed by component PC2 (Fig. 4). It can be confirmed that the fermented and unfermented rice bran samples have different sensory and aroma profiles. While rice bran samples fermented by *K. marxianus* can be characterized by rose aroma. Volatile compounds including phenyl ethyl alcohol, phenyl ethyl acetate, isoamyl acetate and guaiacol are well associated with rose aroma in fermented rice bran samples by *K. marxianus*. On the other hand, fermented cereal, polish and wood aromas well characterized in rice bran samples fermented *D. hansenii* and these aromas was found to related with 2-heptanal, 2,5-dimethyl-3-ethylpyrazine, methanethiol, acetic acid and 2-heptanone presented in the rice bran samples fermented by *D. hansenii* (PC2). In case of unfermented rice samples, boiled corn, boiled wheat porridge, wet bran and dough aromas well characterized in unfermented rice bran and they are associated with 1-octen-3-ol, isobutyl acetate, diacetyl, 2-acetyl-1-pyrroline, 2-acetyl-2-thiazoline and (E)-2-nonenal (PC1).

Due to limited number of sensory studies in fermented rice bran for production of flavor compounds, the findings in the present study could not compared with the literature results. However, aroma production behaviors of *K. marxianus* and *D. hansenii* on tomato and pepper pomace were evaluated in our previous study [11]. Rose aroma was also determined in pepper pomace fermented by *K. marxianus* and *D. hansenii* and a higher wet bulgur aroma observed in tomato pomace fermented by *D. hansenii*. In a study by Kılmanoğlu et al. [43], rose aroma was also determined at high intensity in tomato pomace hydrolysate fermented by *K. marxianus* during 8.5 hours. Similar to our findings, İşleten-Hoşoglu [56] reported that *K. marxianus* produced higher alcohol and acetate esters including isoamyl alcohol, phenyl ethyl alcohol, isoamyl acetate and phenyl ethyl acetate in synthetic culture media supplemented with different carbon and nitrogen sources. The researcher determined that "sourdough", "flower" and "sweet aromatic" were characteristic descriptors for fermented culture media. Sweet aromatic and caramel aromas were found to associate with isoamyl acetate and phenylethyl propionate based on multidimensional scale analysis.

D. hansenii commonly found as natural floral yeast for some surface ripened cheese and dry meat products and it is considered as potential flavor producer [57, 58]. Sørensen et al. [58] reported that strain of *D. hansenii* D18335 primarily produced branched-chain aldehydes and their alcohol derivatives including 2-methylpropanal and 2/3-methylbutanal, 2-methyl-1-propanol and 2/3-methyl-1-butanol in a cheese-surface model. While branched-chain aldehydes produced by *D. hansenii* are associated with green, malty, chocolate, and almond, alcohols are associated fruity, solvent, alcoholic, fusel and pomace aromas. In another study by Leclercq-Perlat et al. [59],

methyl ketones with fruity, floral moldy, cheesy, wine aromas and 2-phenyl ethyl alcohol with rose aroma were determined in deacidified model cheese medium generated by *D. hansenii*. In the present study, 2-methylpropanal and 2/3-methylbutanal and their alcohols derivatives by the metabolism of *D. hansenii* in rice bran were not determined. This difference might be attributed to nature of raw materials used in the studies and strain differences. On the other hand, the production of phenyl ethyl alcohol associated with rose aroma in fermented rice bran were determined in the present study which was similar to the findings of Leclercq-Perlat et al.[59].

Conclusion

Several researchers focused on the potential usage of agro wastes due to their sustainable utilizations for eco-friendly processes and low costs. The observed results in the present study showed that higher alcohols including isoamyl alcohol and phenyl ethyl alcohol and their acetate esters can be produced from rice bran by using *K. marxianus* and *D. hansenii*. The microbial growths of both yeasts in rice bran were determined to range between 2.30–3.69 log CFU/mL depending on fermentation conditions. In bioreactor fermentation, *K. marxianus* can produce 827.27 µg/kg of isoamyl alcohol, 169.77 µg/kg of phenyl ethyl alcohol and 216.08 µg/kg phenyl ethyl acetate during 24 h batch fermentation. Isovaleric acid was also found produced by *K. marxianus* at high level (4013 µg/kg) in the batch fermentation. The highest concentrations of isoamyl alcohol and phenyl ethyl acetate were determined as 415.64 µg/kg and 135.77 µg/kg, respectively at 24 h fermentation by *D. hansenii*. Overall, the rice bran could be valorized for the natural flavor production by biotechnological process. Further studies are required for revealing the flavor production of certain yeasts in different food by-products or agro wastes. Moreover, the studies on modeling of stripping effect in the bioreactor fermentation should also be performed for the microbial production of flavor compounds from rice bran to obtain higher productivity rates.

Declarations

Acknowledgments

The authors would like to thank to Bioflavor COST Action FA0907 for supporting this scientific work. The authors would also like to thank to panelists for their participation and contribution during sensory panels.

Funding

This study was supported by The Scientific and Technological Council of Turkey (TUBITAK, Ankara Turkey; Project No. 1100903).

Declaration of Competing Interest

The authors declare no conflicts of interest.

Ethical Guidelines

Ethics approval was not required for this research.

Data Availability Statement

Research data are not shared. The data generated during the present study are available from the corresponding author upon reasonable request.

References

1. Hosoglu, M.I., Guneser, O., Yuceer, Y.K.: Different bioengineering approaches on production of bioflavor compounds. In: Grumezescu, A.M. and Holban, A.M. (eds.) *Handbook of Food Bioengineering*, pp. 37–71. Academic Press, Cambridge (2018)
2. Scragg, A.H.: The production of flavours by plant cell cultures. In: Berger, R.G. (ed.) *Flavours and Fragrances: Chemistry, Bioprocessing and Sustainability*. pp. 599–614. Springer, Heidelberg (2007)

3. Ziegler, H.: Introduction. In: Ziegler, H. (ed.) Flavourings: Production, Composition, Applications, Regulations, pp.1-13. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim (2007)
4. Bettio, G., Zardo, L.C., Rosa, C.A., Záchia Ayub, M.A.: Bioconversion of ferulic acid into aroma compounds by newly isolated yeast strains of the Latin American biodiversity. *Biotechnol. Prog.*, e3067 (2020). <https://doi.org/10.1002/bmpr.3067>
5. Ben Akacha, N., Gargouri, M.: Microbial and enzymatic technologies used for the production of natural aroma compounds: synthesis, recovery modeling, and bioprocesses. *Food Bioprod. Process.* 94, 675–706 (2015).
6. Coimbra, J.M., Cristina dos Reis, K., Schwan, R.F., Silva, C.F.: Effect of the strategy of molasses supplementation in vinasse to high SCP production and rose flavor compound. *Waste and Biomass Valorization*. (2020). <https://doi.org/10.1007/s12649-020-00961-2>
7. Yilmaztekin, M., Erten, H., Cabaroglu, T.: Enhanced production of isoamyl acetate from beet molasses with addition of fusel oil by *Williopsis saturnus* var. *saturnus*. *Food Chem.* 112, 290–294 (2009).
8. Rossi, S.C., Vandenberghe, L.P.S., Pereira, B.M.P., Gago, F.D., Rizzolo, J.A., Pandey, A., Soccol, C.R., Medeiros, A.B.P.: Improving fruity aroma production by fungi in SSF using citric pulp. *Food Res. Int.* 42, 484–486 (2009)
9. Chakraborty, D., Kaur, B., Obulisanmy, K., Selvam, A., Wong, J.W.C.: Agrowaste to vanillin conversion by a natural *Pediococcus acidilactici* strain BD16. *Environ. Technol.* 38, 1823–1834 (2017)
10. Fadel, H.H.M., Mahmoud, M.G., Asker, M.M.S., Lotfy, S.N.: Characterization and evaluation of coconut aroma produced by *Trichoderma viride* EMCC-107 in solid state fermentation on sugarcane bagasse. *Electron. J. Biotechnol.* 18, 5–9 (2015)
11. Güneşer, O., Demirkol, A., Karagül Yüceer, Y., Özmen Toğay, S., İşleten Hoşoğlu, M., Elibol, M.: Bioflavour production from tomato and pepper pomaces by *Kluyveromyces marxianus* and *Debaryomyces hansenii*. *Bioprocess Biosyst. Eng.* 38, 1143–1155 (2015)
12. Güneşer, O., Yüceer, Y.K.: Biosynthesis of eight-carbon volatiles from tomato and pepper pomaces by fungi: *Trichoderma atroviride* and *Aspergillus sojae*. *J. Biosci. Bioeng.* 123, 451–459. (2017)
13. Wang, M., Lei, M., Samina, N., Chen, L., Liu, C., Yin, T., Yan, X., Wu, C., He, H., Yi, C.: Impact of *Lactobacillus plantarum* 423 fermentation on the antioxidant activity and flavor properties of rice bran and wheat bran. *Food Chem.* 330, 127156 (2020). <https://doi.org/https://doi.org/10.1016/j.foodchem.2020.127156>
14. Yılmaz Tuncel, N., Yılmaz Korkmaz, F.: Comparison of lipid degradation in raw and infrared stabilized rice bran and rice bran oil: matrix effect. *J. Food Meas. Charact.* (2020). <https://doi.org/10.1007/s11694-020-00709-5>
15. Sharif, M.K., Butt, M.S., Anjum, F.M., Khan, S.H.: Rice Bran: A Novel Functional Ingredient. *Crit. Rev. Food Sci. Nutr.* 54, 807–816 (2014)
16. Zheng, L., Zheng, P., Sun, Z., Bai, Y., Wang, J., Guo, X.: Production of vanillin from waste residue of rice bran oil by *Aspergillus niger* and *Pycnoporus cinnabarinus*. *Bioresour. Technol.* 98, 1115–1119 (2007)
17. Atlas, R.M.: *Handbook of Microbiological Media*. CRC Press, Boca Raton, Florida, USA (2004)
18. Avsar, Y.K., Karagul-Yuceer, Y., Drake, M.A., Singh, T.K., Yoon, Y., Cadwallader, K.R.: Characterization of nutty flavor in Cheddar cheese. *J. Dairy Sci.* 87, 1999–2010 (2004)
19. Meilgaard, M., Civille, G., Carr, B.: *Sensory evaluation techniques*. CRC Press LLC, Boca Raton, Florida, USA (1999)
20. Sheskin, D.: *Handbook of parametric and nonparametric statistical procedures*. New York: Chapman and Hall /CRC press (2004)
21. Wilkowska, A., Kriegel, D., Guneser, O., Karagul Yuceer, Y.: Growth and by-product profiles of *Kluyveromyces marxianus* cells immobilized in foamed alginate. *Yeast.* 32, 217–225. (2015).
22. Zafar, S., Owais, M.: Ethanol production from crude whey by *Kluyveromyces marxianus*. *Biochem. Eng. J.* 27, 295–298 (2006)
23. Goshima, T., Tsuji, M., Inoue, H., Yano, S., Hoshino, T., Matsushika, A.: Bioethanol production from lignocellulosic biomass by a novel *Kluyveromyces marxianus* strain. *Biosci. Biotechnol. Biochem.* 77, 1505–1510 (2013).
24. Mardawati, E., Andoyo, R., Muhaemin, M., Nurjanah, S., Natawigena, D., Herwanto, T., Handarto, Utama, G.L., Rosalinda, Poppy, L., Kramadibrata, A.M.: Fermentation Process of Glycerol to Arabitol from Byproducts of *Reutalis trisperma* Biodiesel Using Yeast of *Debaryomyces hansenii*. *IOP Conf. Ser. Earth Environ. Sci.* 347, (2019).
25. Sharma, N.K., Behera, S., Arora, R., Kumar, S.: Evolutionary Adaptation of *Kluyveromyces marxianus* NIKE-K3 for Enhanced Xylose Utilization. *Front. Energy Res.* 5:32. (2017)
26. Maurya, D.P., Singla, A., Negi, S.: An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol. *3 Biotech.* 5, 597–609 (2015)
27. Singh nee' Nigam, P., Gupta, N., Anthwal, A.: Pre-treatment of agro-industrial residues. In: Singh nee' Nigam, P. and Pandey A. (eds.) *Biotechnology for Agro-Industrial Residues Utilisation*, pp 13-33. Springer, Heidelberg (2009)

28. Drake, M.A., Civille, G. V: Flavor Lexicons. *Compr. Rev. Food Sci. Food Saf.* 2, 33–40 (2003)
29. Czerny, M., Christlbauer, M., Christlbauer, M., Fischer, A., Granvogl, M., Hammer, M., Hartl, C., Hernandez, N.M., Schieberle, P.: Investigation on odour thresholds of key food aroma compounds and development of an aroma language based on odour qualities of defined aqueous odorant solutions. *Eur. Food Res. Technol.* 228, 265–273 (2008)
30. Koehler, P.E., Mason, M.E., Odell, G. V: Odor threshold levels of pyrazine compounds and assessment of their role in the flavor of roasted foods. *J. Food Sci.* 36, 816–818 (1971)
31. Belitz, H.-D., Grosch, W., Schieberle, P: *Food Chemistry*. Springer, Heidelberg (2009)
32. Saerens, S.M.G., Delvaux, F.R., Verstrepen, K.J., Thevelein, J.M.: Production and biological function of volatile esters in *Saccharomyces cerevisiae*. *Microb. Biotechnol.* 3, 165–177 (2010)
33. Bartowsky, E.J., Pretorius, I.S.: Microbial formation and modification of flavor and off-flavor compounds in wine. In: König, H., Unden, G., Fröhlich J.H (eds.) *Biology of Microorganisms on Grapes, in Must and in Wine*. pp. 209-231. Springer, Heidelberg (2009)
34. Hazelwood, L.A., Daran, J.M., Van Maris, A.J.A., Pronk, J.T., Dickinson, J.R.: The Ehrlich pathway for fusel alcohol production: A century of research on *Saccharomyces cerevisiae* metabolism. *Appl. Environ. Microbiol.* 74, 2259–2266 (2008)
35. Etschmann, M., Bluemke, W., Sell, D., Schrader, J.: Biotechnological production of 2-phenylethanol. *Appl. Microbiol. Biotechnol.* 59, 1–8 (2002)
36. Martínez-Avila, O., Sánchez, A., Font, X., Barrena, R.: Bioprocesses for 2-phenylethanol and 2-phenylethyl acetate production: current state and perspectives. *Appl. Microbiol. Biotechnol.* 102, 9991–10004 (2018).
37. Varela, J.A., Gethins, L., Stanton, C., Ross, P., Morrissey, J.P.: Applications of *Kluyveromyces marxianus* in biotechnology. In: Satyanarayana, T., Kunze G. (eds.) *Yeast Diversity in Human Welfare*, pp. 439-453. Springer, Heidelberg (2017)
38. Gethins, L., Guneser, O., Demirkol, A., Rea, M.C., Stanton, C., Ross, R.P., Yuceer, Y., Morrissey, J.P.: Influence of carbon and nitrogen source on production of volatile fragrance and flavour metabolites by the yeast *Kluyveromyces marxianus*. *Yeast* 32, 67-76 (2015)
39. Van Der Sluis, C., Rahardjo, Y.S.P., Smit, B.A., Kroon, P.J., Hartmans, S., Ter Schure, E.G., Tramper, J., Wijffels, R.: Concomitant extracellular accumulation of alpha-keto acids and higher alcohols by *Zygosaccharomyces rouxii*. *J. Biosci. Bioeng.* 93, 117–124 (2002)
40. Chatonnet, P., Dubourdieu, D., Boidron, J. -n, Lavigne, V.: Synthesis of volatile phenols by *Saccharomyces cerevisiae* in wines. *J. Sci. Food Agric.* 62, 191–202 (1993)
41. Shinohara, T., Kubodera, S., Yanagida, F.: Distribution of phenolic yeasts and production of phenolic off-flavors in wine fermentation. *J. Biosci. Bioeng.* 90, 90–97 (2000)
42. Hübschmann, H.J.: *Handbook of GC-MS Fundamentals and Applications* Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim (2015)
43. Kilmanoğlu, H., Hoşoğlu, M.İ., Güneşer, O., Yüceer, Y.K.: Optimization of pretreatment and enzymatic hydrolysis conditions of tomato pomace for production of alcohols and esters by *Kluyveromyces marxianus*. *LWT*. 138, 110728 (2021)
44. Vong, W.C., Liu, S.Q.: Changes in volatile profile of soybean residue (okara) upon solid-state fermentation by yeasts. *J. Sci. Food Agric.* 97, 135–143 (2017)
45. Lalou, S., Mantzouridou, F., Paraskevopoulou, A., Bugarski, B., Levic, S., Nedovic, V.: Bioflavour production from orange peel hydrolysate using immobilized *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* 97, 9397–9407 (2013)
46. Mantzouridou, F., Paraskevopoulou, A.: Volatile Bio-ester Production from Orange Pulp-Containing Medium Using *Saccharomyces cerevisiae*. *Food Bioprocess Technol.* 6, 3326–3334 (2013)
47. Yilmaztekin, M., Cabaroglu, T., Erten, H.: Effects of Fermentation Temperature and Aeration on Production of Natural Isoamyl Acetate by *Williopsis saturnus* var. *saturnus*. *Biomed Res. Int.* 2013, 870802 (2013)
48. Martínez, O., Sánchez, A., Font, X., Barrena, R.: Valorization of sugarcane bagasse and sugar beet molasses using *Kluyveromyces marxianus* for producing value-added aroma compounds via solid-state fermentation. *J. Clean. Prod.* 158, 8–17 (2017)
49. Urit, T., Löser, C., Wunderlich, M., Bley, T.: Formation of ethyl acetate by *Kluyveromyces marxianus* on whey: studies of the ester stripping. *Bioprocess Biosyst. Eng.* 34, 547–559 (2011)
50. Medeiros, A.B.P., Pandey, A., Vandenberghe, L.P.S., Pastore, G.M., Soccol, C.R.: Production and recovery of aroma compounds produced by solid-state fermentation using different adsorbents. *Food Technol. Biotechnol.* 44, 47–51 (2006)
51. Medeiros, A.B.P., Pandey, A., Christen, P., Fontoura, P.S.G., de Freitas, R.J.S., Soccol, C.R.: Aroma compounds produced by *Kluyveromyces marxianus* in solid state fermentation on a packed bed column bioreactor. *World J. Microbiol. Biotechnol.* 17, 767–771 (2001)

52. Yilmaztekin, M., Erten, H., Cabaroglu, T.: Production of Isoamyl Acetate from Sugar Beet Molasses by *Williopsis saturnus* var. *saturnus*. J. Inst. Brew. 114, 34–38 (2008)
53. Lukin, I., Merz, J., Schembecker, G.: Techniques for the recovery of volatile aroma compounds from biochemical broth: A review. Flavour Fragr. J. 33, 203–216 (2018)
54. Try, S., Voilley, A., Chunhieng, T., De-Coninck, J., Waché, Y.: Aroma compounds production by solid state fermentation, importance of in situ gas-phase recovery systems. Appl. Microbiol. Biotechnol. 102, 7239–7255 (2018)
55. Tay, S.: Using the method of “In Situ Product Removal” while producing natural banana flavor through bioconversion. (Master Thesis), İnonü University, Graduate School of Natural and Applied Sciences, Malatya, Turkey (2013)
56. İşleten Hoşoğlu, M.: Study of increasing the production of volatile flavor compounds by the yeast *Kluyveromyces marxianus* through optimization of carbon and nitrogen sources. Food Health. 4, 112–123 (2018)
57. Cano-García, L., Flores, M., Belloch, C.: Molecular characterization and aromatic potential of *Debaryomyces hansenii* strains isolated from naturally fermented sausages. Food Res. Int. 52, 42–49 (2013)
58. Sørensen, L.M., Gori, K., Petersen, M.A., Jespersen, L., Arneborg, N.: Flavour compound production by *Yarrowia lipolytica*, *Saccharomyces cerevisiae* and *Debaryomyces hansenii* in a cheese-surface model. Int. Dairy J. 21, 970–978 (2011)
59. Leclercq-Perlat, M.N., Corrieu, G., Spinnler, H.E.: Comparison of volatile compounds produced in model cheese medium deacidified by *Debaryomyces hansenii* or *Kluyveromyces marxianus*. J. Dairy Sci. 87, 1545–1550 (2004)

Figures

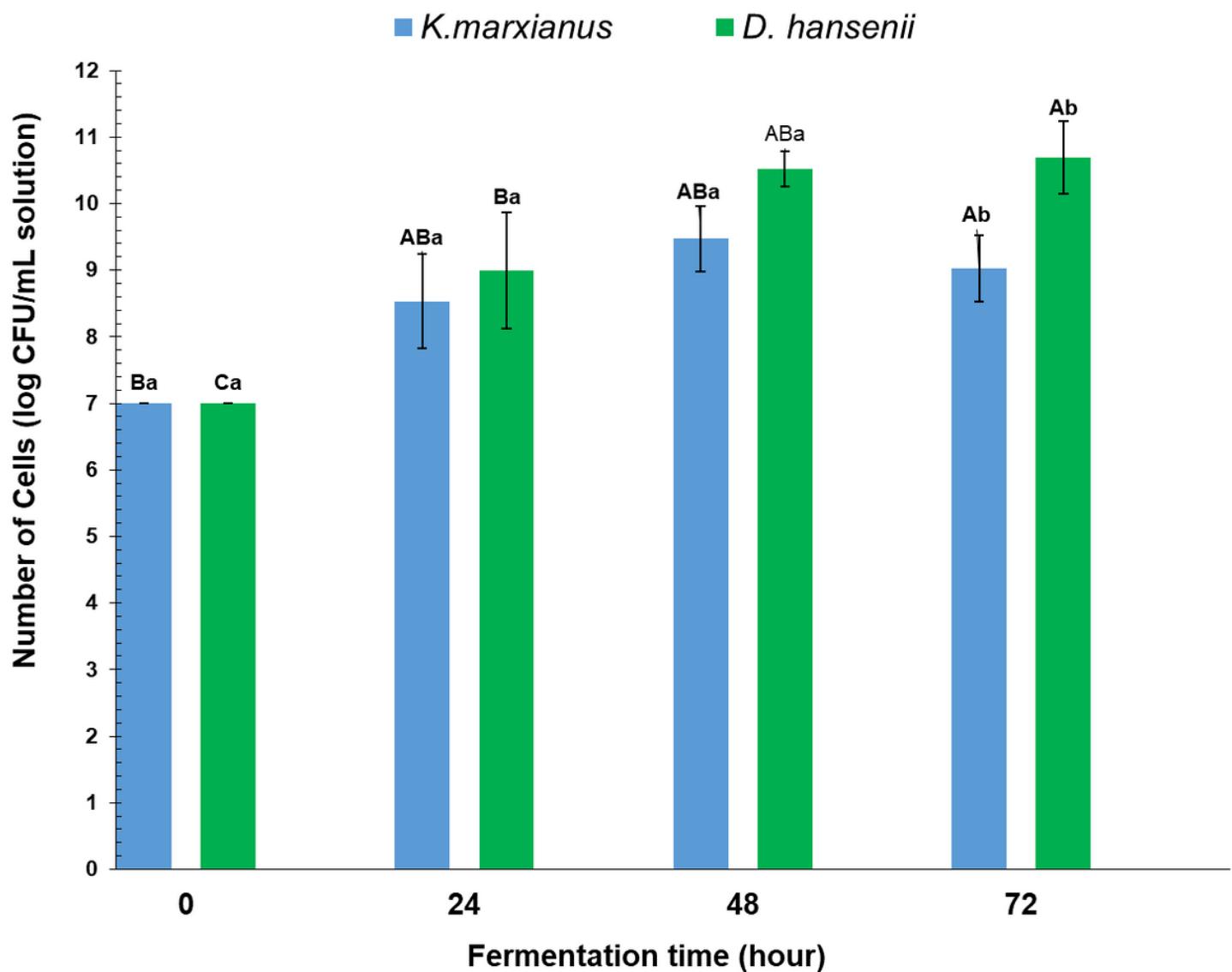


Figure 1

Microbial growth curve of *K. marxianus* and *D. hansenii* in the shake flask fermentation. A–B Bars not sharing common uppercase letters are different for the fermentation of *K. marxianus*. a–bBars not sharing common lowercase letters are different for the fermentation of *D. hansenii*, $p \leq 0.05$)

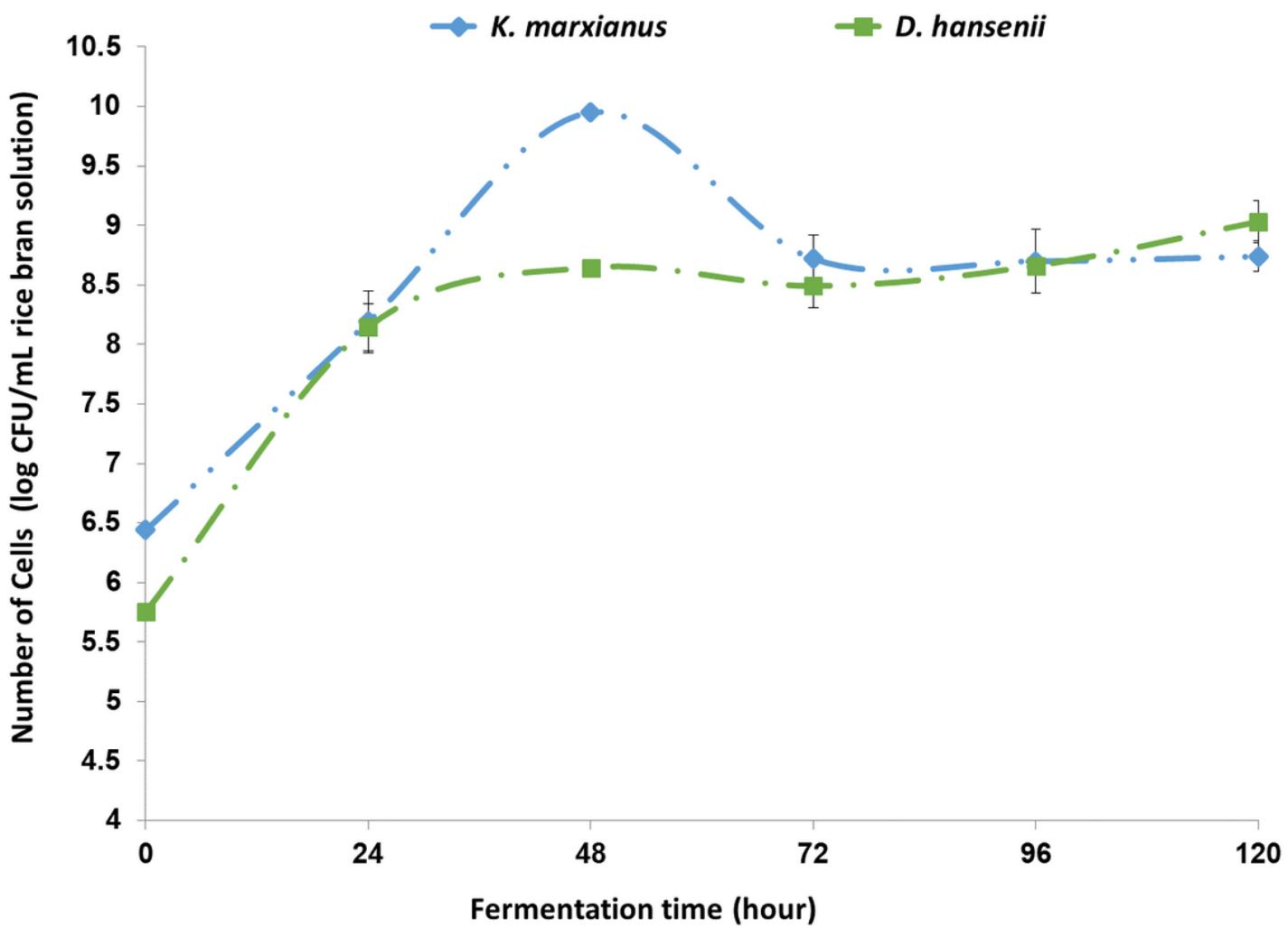


Figure 2

Microbial growth curve of *K. marxianus* and *D. hansenii* in the shake flask fermentation. A–B Bars not sharing common uppercase letters are different for the fermentation of *K. marxianus*. a–b Bars not sharing common lowercase letters are different for the fermentation of *D. hansenii*, $p \leq 0.05$

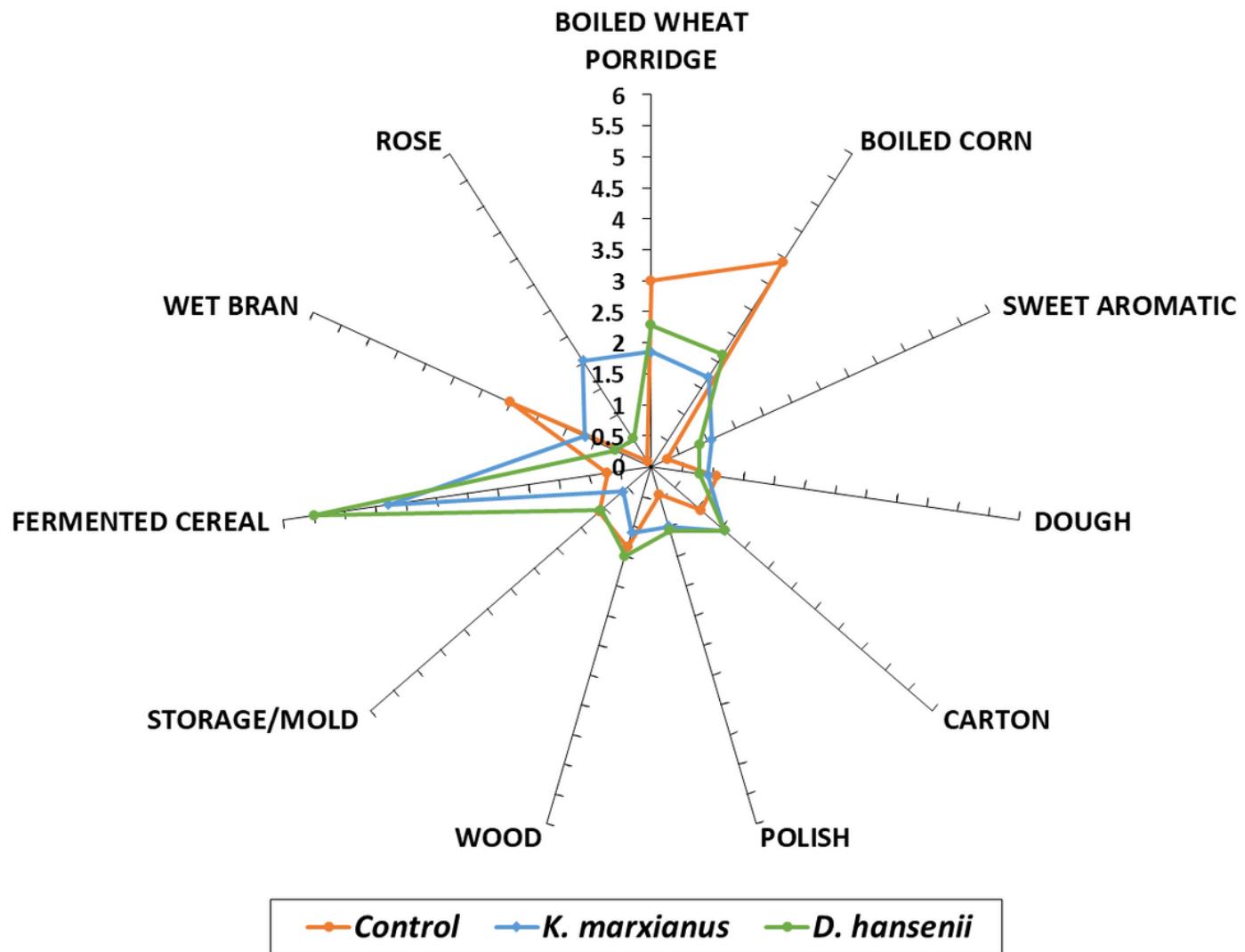


Figure 3

Spider diagram for the descriptive flavor terms of the rice bran fermented by *K. marxianus* and *D. hansenii* (bulgur: cracked wheat)

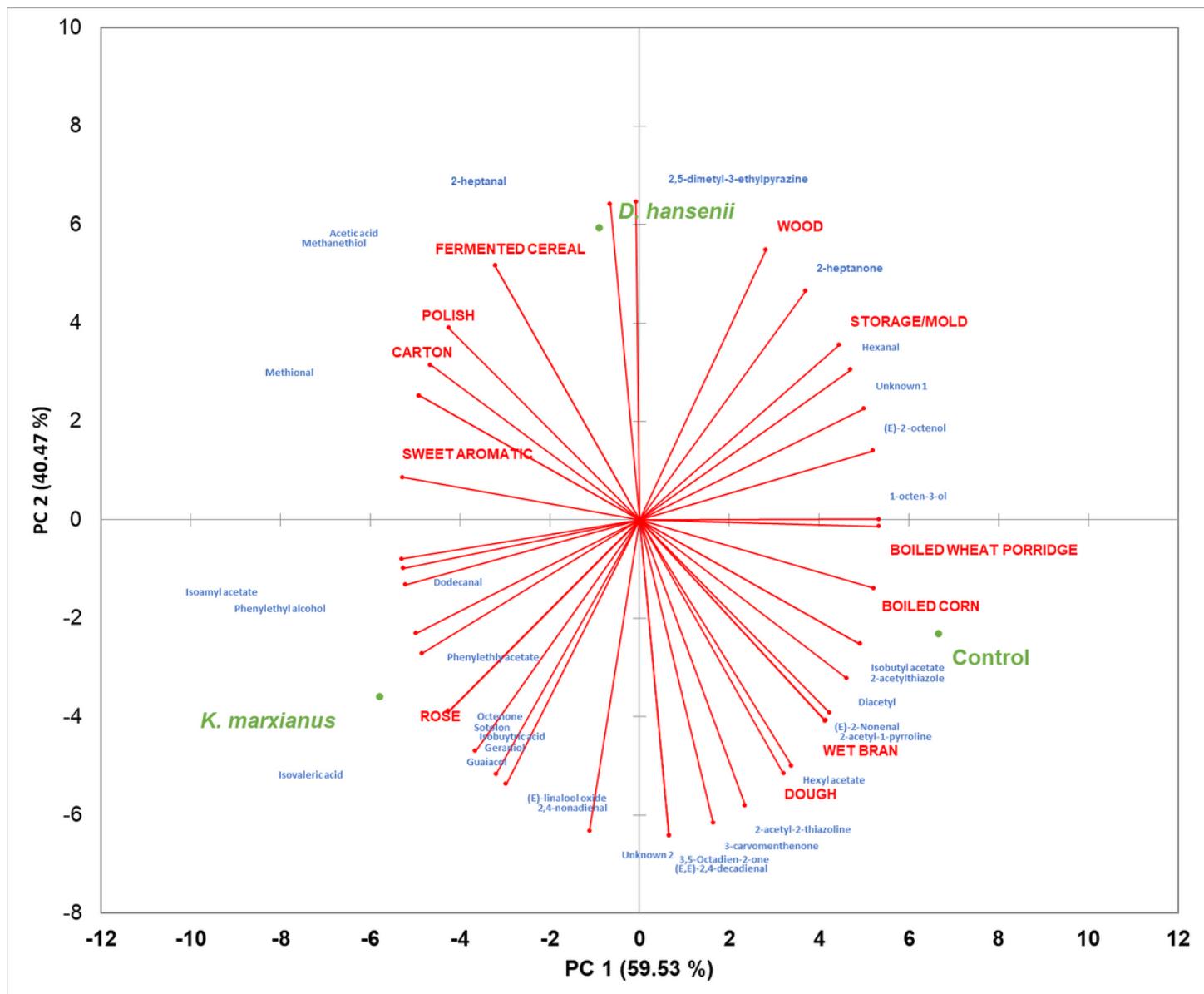


Figure 4

Relationship between aroma-active compounds and sensory properties of the rice bran samples.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- graphicalababstractson.png