

Aroma of Yogurt from Cow Milk with a Combination of Several Probiotic Bacteria

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ABSTRACT

This study aimed to investigate the effect of different probiotic bacteria on aroma of yogurt through a unique incubation process. Probiotic bacteria examined were *Lactobacillus*, *Streptococcus*, and *Bifidobacterium*. Additionally, yogurt production process included sterilizing skim milk, introducing bacterial cultures, and incubating at specific temperatures. Aroma components of yogurt were analyzed using gas chromatography and mass spectrometry. The result showed that yogurt comprised various aroma compounds classified into four categories, namely, acids, alcohols, ketones, and aldehydes. The dominant acid components found were acetic, octanoic, decanoic, and dodecanoic acids. Among the components, alcohol affected aroma despite being present in minimal quantities. Ketones such as acetoin and diacetyl were identified along with aldehydes including octadecanal and dodecanal in yogurt. This study provided valuable insights into the effect of probiotic bacteria on aroma profile of yogurt, assisting manufacturers in refining production processes to prepare product appealing to consumers.

Keywords: Aroma compounds, Gas chromatography, Mass spectrometry, Yogurt production.

INTRODUCTION

Yogurt is a popular fermented milk product known for the nutritional value and unique aroma, which varies depending on the used ingredients. The major ingredient in yogurt is primarily cow milk, and live cultures such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are commonly used during the production. Additional flavorings or sweeteners may be added to customize the taste of yogurt (Nair and Peerkhan, 2022; Trentin *et al.*, 2022; Wihansah *et al.*, 2022; Fadhlurrohman *et al.*, 2023).

Aroma of yoghurt plays a crucial role in the acceptance by consumers and this varies significantly based on factors such as the type of probiotic bacteria used in fermentation, milk type, and the incubation methods applied. According to Chen (2017), yogurt production is a complex process that

generates a distinctive aroma. Zhao *et al.* (2023) reported that factors such as the production process, extraction method, starter culture concentration, and incubation time could further affect aroma.

Aktar (2022) stated that aroma produced was affected by variations in the use of bacteria. Krastanov *et al.* (2023) found that the starter culture (bacteria) affected the sensory characteristics, including aroma and taste of yogurt.

A key factor in shaping the preferences of consumers for yogurt product is aroma, while the perceptions greatly determine purchase decisions and continual product enjoyment. This shows the need to comprehend the various aroma components of yogurt as well as the effect on consumers' preferences in the food and beverage industry. Aroma is affected by several factors, including the type of bacteria in the starter culture, the

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processing method, the source of milk and chemicals, and any additional ingredients used. According to Eker *et al.* (2020), aroma is essential in shaping the preferences of consumers and the general sensory experience of yogurt.

Probiotic is a group of bacteria commonly found in fermented milk product that provides numerous health benefits to humans. According to Latif *et al.* (2023), probiotic is live microorganism that offers health advantages to the host when consumed in sufficient quantities. The seven primary microbial genera majorly used in probiotic product are *Lactobacillus*, *Bifidobacterium*, *Saccharomyces*, *Streptococcus*, *Enterococcus*, *Escherichia*, and *Bacillus*.

Probiotic bacteria used in this study were *Lactobacillus*, *Streptococcus*, and *Bifidobacterium*, which provide health benefits and produce various aroma components. The combination of the three bacteria is expected to help with the production of high-quality yogurt due to containing a harmonious blend. According to Siddiqi *et al.* (2024), *L. bulgaricus* and *S. thermophilus* bacteria contribute to a decrease in pH, which is important for the texture and flavor of yogurt. *Bifidobacteria* is among the dominant colonies in the human gut, specifically in infants (He *et al.*, 2023; Znamenska and Vorobiova, 2024). Kamara *et al.* (2016) reported that yogurt fermented with mixed cultures of *L. bulgaricus*, *S. thermophilus*, and *L. acidophilus* showed antibacterial activity against *E. coli* and *Bacillus subtilis*. A study by Elbarbary (2014) found that the inclusion of bifidobacteria spp. in stored yogurt inhibited the growth of *E. coli* and *Staphylococcus aureus*.

During yogurt fermentation, probiotic bacteria used are essential in developing the unique aroma of the finished product and various strains can generate different aroma compounds. Therefore, selecting the appropriate probiotic combination is crucial in achieving the desired aroma.

Several previous investigations focused on different culture treatments and temperature variations (Yu *et al.*, 2016; Swelam *et al.*,

2019; Jegal *et al.*, 2019; Adrianto *et al.*, 2020). However, a unique method was used in this study where *Bifidobacteria* was incubated with *L. bulgaricus* and *S. thermophilus* cultures, as well as in a separate manner.

This study aimed to examine the effect of various probiotic bacteria on yogurt aroma. Understanding the effect of microbial factors can help yogurt producers enhance manufacturing processes to prepare product with an appealing aroma that resonates with consumers. Aroma profile in this study is determined by the presence of volatile components.

MATERIALS AND METHODS

Material and Equipment

Commercial powdered skim milk was used in yogurt production, along with bacteria strains *L. bulgaricus* 18 Visbyvac Serie 50 No. 700398 (*L. bulgaricus*), *S. thermophilus* A Vysbivac No. 640638 (*S. thermophilus*), and *Bifidobacterium longum* BF1 (*B. longum*). Additionally, the laboratory equipment used were a rotary evaporator, gas chromatograph (Shimadzu 17 A), and mass spectrometer (Shimadzu QP 5000), as well as Erlenmeyer tubes and other necessary glass tools.

Yogurt Preparation and Production

Skim milk powder was dissolved in distilled water until a specific gravity of 1.027 equivalent to fresh milk was attained. The mixture was sterilized at 121°C for 15 minutes in an autoclave. After cooling to 42°C, a commercial culture of *L. bulgaricus*/*S. thermophilus* was added at a concentration of 2% of the sterilized milk volume. The resulting mixture was incubated at 37°C for 20 hours, then, cooled to 37°C before adding *B. longum* to the milk. *B. longum* was selected over more commonly used probiotic due to the superior adaptability and health

benefits provided in the human gut (Sharma *et al.*, 2021; Li *et al.*, 2023; Li *et al.*, 2024).

Preparing Samples for the Analysis of Aromatic Compounds

The separation of volatile aroma components is conducted using the Likens-Nickerson equipment and SDE (Simultaneous Distillation Extraction) method. A Gas Chromatograph was used in combination with a Mass Spectrometer (GC-MS) to analyze aroma components following the method described by Shiratsuchi *et al.* (1994). A solution of 300 g yogurt dissolved in 900 mL distilled water was used to separate aroma components, with polyethylene glycol 4000 added to prevent foaming. Subsequently, extraction and distillation were carried out simultaneously for 1 hour using 30 mL of diethyl ether as the solvent. Anhydrous sodium sulfate was added to bind water, and the solution was concentrated with a rotary evaporator.

Nitrogen gas was used to obtain the distillate, which was injected into GC-MS apparatus. This process was duplicated and analyzed under the conditions presented in Table 1.

Yogurt was produced in this study using the incubation method with a freeze-dried *B. longum* bacterial culture. The incubation process of the culture was carried out for 20 hours at 37°C.

Seven combination treatments were performed using probiotic bacteria and the incubation method during yogurt production. These included the followings:

- 1) L= Single culture of *L. bulgaricus*,
 - 2) LB1= *L. bulgaricus*+*B. longum* incubated,
 - 3) S= Single culture of *S. thermophilus*,
 - 4) SB1= *S. thermophilus*+*B. longum* incubated,
 - 5) LS= *L. bulgaricus*+*S. thermophilus*,
 - 6) LSB1= *L. bulgaricus*+*S. thermophilus*+*B. longum* incubated,
 - 7) B= Single culture of *B. longum*,
- The results are reported descriptively.

Table 1. Condition of GC-MS Instrument Used.

Condition of GC-MS	Information
GC Conditions	
Brand of tools	: Shimadzu Model 17 A
Column type	: Shimadzu CBP20-M50_025. Column length 50 m (i.d. 0.22 mm and o.d. 0.33 mm). Hi-Cap series. Film layer thickness 0.25 µm
Carrier gas	: Helium, pressure 199 kPa
Initial temperature	: 50°C duration of 5 minutes
Final temperature	: 200°C duration of 30 minutes
The rate of temperature increase	: 4°C per minute
Sample volume.	: 1 µl
Injection method	: Grob Split-splless; Sampling time is 30 seconds
Temperature interface	: 230°C
Injector temperature	: 220°C
MS Conditions	
Brand of tools	: Shimadzu model QP 5000
Energy detector	: 1.1 kV
Mass range	: 33 – 400
Resolution	: 1000
Database.	: National Institute for Standard and Technology (NIST) library



RESULTS AND DISCUSSION

Yogurt manufactured contains a wide variety of aroma components grouped into acids, alcohols, ketones, and aldehydes. This study found that the entire components vary greatly in both quantity and type.

Group of Acids

The results showed that yogurt group had a dominant presence of acids in terms of aroma components. GC-MS analysis can be used to identify various acidic compounds, specifically volatile acids that evaporate when exposed to high GC temperatures and pressures. Table 2 presents the acidic compounds detected in the treated yogurt.

Yogurt is a dairy product containing various acids, such as lactic acids essential for the taste and texture. During fermentation process, Lactic Acid Bacteria (LAB), such as *L. bulgaricus* and *S. thermophilus*, consume the lactose in milk and convert it into lactic

acids. The tangy flavor of yogurt originates from lactic acids, which also helps prevent the growth of harmful bacteria and contributes to the thick product consistency. The performance of LAB starters directly affects the quality of yogurt, including the texture and gel properties (Zhang and Zhang, 2012). LAB from traditionally prepared dairy product, such as *Lactobacillus plantarum*, was found to enhance the condensed and textural integrity of yogurt (Li et al., 2022).

Acids are long carbon chain fat molecules with a carboxylic acid group at the terminal portion. The milk used in yogurt production contains both saturated and unsaturated acids, which contribute to the characteristic taste and aroma. Additionally, acids play a role in the thickness and texture of yogurt. Some acids, such as butyric (butanoic) and caproic (octanoic) acids, can impart a buttery taste to yogurt, while others, including acetic and hexanoic acids, contribute to a soft and fruity aroma. According to Zhao et al. (2023) and Tian et al. (2019), aroma is influenced by various acids, including butyric, caproic,

Table 2. Group of acids.^a

No.	Types of acids	RT	LRI	LRI (Ref.)	Treatments						
					L	LB	S	SB	LS	LSB	B
1	Acetic acid	21.60	1457	1425*	1.37	2.11	0.87	5.95	3.06	3.28	2.04
2	Butanoic acid	27.30	1631	1652*	2.08	1.55	0.38	1.12	3.66	3.58	4.56
3	Hexanoic acid	33.60	1842	1849*	6.63	7.98	1.26	10.59	9.88	9.06	10.5
4	Octanoic acid	38.02	2010	2084*	19.72	20.22	6.73	15.11	22.9	20.35	16.5
5	Nonanoic acid	41.95	2166	2192*	0.68	0.99	0.33	0.47	0.82	2.92	0.41
6	Decanoic acid	44.88	>2200	2486*	20.72	19.94	18.7	14.95	14.6	15.38	14.4
7	Benzoic acid	50.83	>2200	2380*	0.15	1.81	0	0	0.16	1.12	0.32
8	Dodecanoic acid	52.15	>2200	2517**	1.06	3.79	27.7	10.4	0	0	20.3
9	Undecanoic acid	53.28	>2200	2365***	1.48	6.19	0.24	9.8	5.52	3.06	0.28
10	Tetradecanoic acid	64.11	>2200	2733**	3.06	1.03	13.4	5.25	0	0	8.91

^a L= Yogurt with single *L. bulgaricus* culture; LB= Yogurt with *L. bulgaricus* and *B. longum* cultures; S= Yogurt with single *S. thermophilus* culture; SB= Yogurt with *S. thermophilus*+*B. longum* cultures; LS= Yogurt with *L. bulgaricus*+*S. thermophilus* cultures; LSB= Yogurt with *L. bulgaricus*+*S. thermophilus*+*B. longum* cultures; B= Yogurt with single *B. longum* culture; RT= Retention Time; LRI= Linear Retention Index; Ref= Reference.

* Tian et al., 2019; ** Shiratsuchi et al., 1994; *** Marce et al., 1976.

acetic, and hexanoic acids, which collectively contribute to the general flavor profile and sensory characteristics of yogurt.

Analysis of yogurt using GC-MS did not detect lactic acids, potentially due to the hydrophilic nature and presence in the form of ions in yogurt. GC-MS is more effective for detecting volatile non-polar compounds, leading to the unsuitability for detecting lactic acids. This result corresponded with reports from other studies (Zhao *et al.*, 2023; Liu *et al.*, 2022) that failed to detect lactic acids in yogurt.

The data in Table 2 show that the composition of acids varies across the different treatments, suggesting that each treatment has a unique pattern in terms of the type and concentration of acids. Some treatments are characterized by a high concentration of specific types of acids.

Yogurt aroma analysis in this study found that acetic acids were the first to appear, therefore, they are considered as the most volatile component (Zhao *et al.*, 2023) detected using GC-MS (Arslaner, 2020; Yüksel and Bakırcı, 2015; Cheng, 2010). According to Cheng (2010), acetic acids contribute to the vinegar-like, pungent, and acidic aroma of yogurt.

Table 2 shows that certain yogurt using different cultures and combinations (L, LB, B) produce 10 types of volatile acid

SB, LS, and LSB, generated fewer than 10 types of volatile acids. In this study, yogurt with a single culture of *L. bulgaricus* produced 11 acid group compounds. Liu *et al.* (2022) identified 12 aroma-active compounds in yogurt, including butanoic, acetic, and hexanoic acids, as the main aroma-active compounds in fermented milk produced by *L. bulgaricus*. The results showed that yogurt produced with a single culture of *L. bulgaricus* obtained these compounds. Treatments with a single culture of *S. thermophilus* (S), as well as incubation with *L. bulgaricus* (LS) and Bifidobacteria (SB), did not produce benzoic acid. According to Yu *et al.* (2016), benzoic acid production has been studied for various starter cultures and incubation temperatures without a report on the optimization process. The use of a single culture of Bifidobacteria in preparing yogurt produces 10 types of components, among which the most prominent are octanoic, decanoic, and dodecanoic acids. Tian *et al.* (2019) found that both octanoic and decanoic acids contributed to the distinctive taste of cheese, while octanoic acids produced a light creamy taste in yogurt.

The majority of treatments generated high levels of octanoic, decanoic, and dodecanoic compounds compared to the other groups. These acids are present in higher quantities in

Table 3. Group of alcohol.^a

No.	Types of alcohol	RT	LRI	LRI (Ref)*	Treatments						
					L	LB	S	SB	LS	LSB	B
1	2-Nonanol	22.87	1494	1097	2.06	1	0	0	1.12	0.8	0
2	2-Undecanol	29.38	1704	1301	5.06	1.1	0	0	1.89	0.9	0
3	2-Tridecanol	34.81	1885	1570	0	1.04	0	0	1.11	0	0
4	2-Decanol	43.62	2200	1198	2.15	1.41	0	0	0.79	0	0

^a L= Yogurt with single *L. bulgaricus* culture; LB= Yogurt with *L. bulgaricus* and *B. longum* cultures; S= Yogurt with single *S. thermophilus* culture; SB= Yogurt with *S. thermophilus*+*B. longum* cultures; LS= Yogurt with *L. bulgaricus*+*S. thermophilus* cultures; LSB= Yogurt with *L. bulgaricus*+*S. thermophilus*+*B. longum* cultures; B= Yogurt with single *B. longum* culture; RT= Retention Time; LRI= Linear Retention Index; Ref= Reference. *= Adams (2007).

components. Some treatments, including S,

yogurt due to the activity of probiotic bacteria



commonly used during fermentation. Probiotic bacteria, such as *Lactobacillus* and *Bifidobacterium*, convert lactose (milk sugar) into octanoic, decanoic, and dodecanoic acids. Barros *et al.* (2019) stated that the process of converting lactose into acids in yogurt included several steps. Fermentation of lactose leads to the production of acids, which contribute to the flavor and texture of yogurt.

Group of Alcohol

During the preparation of yogurt, microbes ferment lactose and produce acids as well as other compounds, including alcohol. The amount of alcohol produced is extremely low and not considered harmful to health. Additionally, the presence of alcohol can contribute to the unique aroma of yogurt. Hussain *et al.* (2010) found that *S. thermophilus* and *L. bulgaricus* produced alcohol-related compounds as well as saturated volatile free acids during yogurt manufacturing.

Alcohol compounds produced were very low, probably due to acids generated as the main product by bacteria during yogurt preparation, while more alcohol was obtained from fermentation with yeast. Nurhayati *et al.* (2022) found that yeast could generate alcohol, and several studies focused on the isolation and identification of various yeast species with the potential to produce ethanol. The profile of alcohol amount obtained from this study had the same pattern, where some treatments generated a high content of alcohol compounds. The three treatments that produced higher alcohol compounds included LB1, LS, and B.

Group of Ketones, Aldehydes, Furan Derivatives, and Ester Derivatives

Ketones are a type of compound included in the carbonyl group and not the major product formed during yogurt fermentation process. Table 4 shows that acetoin and

diacetyl are the most abundant compounds produced in ketone group.

Acetoin and diacetyl are both ketone compounds commonly used in foods and drinks to enhance specific flavors and aroma. For example, acetoin can impart a buttery taste to yogurt, while diacetyl provides an acidic or buttery flavor. This study showed that all treated yogurt produced acetoin, and diacetyl was only generated by six (L, S, SB, LS, LSB, B) out of the seven treatments. These two are the primary aroma compounds in yogurt playing a significant role in determining the general aroma and taste (Tian *et al.*, 2020). Acetoin is produced by *L. acidophilus* during fermentation, while diacetyl is formed through the lipolysis of milk fat as well as the microbiological transformation of lactose and citrate (Cheng, 2010). The odor threshold for acetoin and diacetyl in yogurt is higher than in water (Nadal *et al.*, 2009). Acetoin is commonly found in food fermentation product due to being a metabolic byproduct of microorganisms such as lactic acid bacteria. In yogurt, acetoin is typically present in higher concentrations than other ketone compounds, leading to easy detection with GC-MS and a more prominent appearance. This is attributed to acetoin being a compound produced by the metabolism of LAB commonly used in the production of yogurt.

Aldehydes are included in the group of carbonyl compounds, along with ketones. The main difference between ketones and aldehydes depends on the atoms attached to the carbonyl group. In ketones, the carbonyl group is bonded to two carbon atoms, while in aldehydes, it is bonded to one carbon and a hydrogen atom. Aldehydes are present in very small amounts as by-product during fermentation process, but not considered a major component or the main characteristic of yogurt.

Table 4. Groups of ketone compounds, aldehydes, furan derivatives, and ester derivatives.^a

No.	RT	LRI	LRI (Ref.)	Compound type	Treatments							
					L	LB	S	SB	LS	LSB	B	
					% area							
A				Ketones								
1	6.09	1014	981*	Diacetyl	0.07	0	2.14	1.7	0.9	1.34	2.4	
2	8.17	1089	1056*	2,3-pentanedione	0.06	0	0.67	0.89	0	0	0.4	
3	15.2	1280	1299*	Acetoin	1.6	3.44	3.4	19.5	2.2	8.38	4.6	
4	18.2	1362	1387*	2-nonanone	0.07	1.63	0	1	1.3	0	0	
5	18.4	1368		3-hydroxi-2-pentanone	0	0	0.27	0.91	0.9	0.61	0.4	
6	31.7	1774	1488*	2-tridecanone	1.56	0.54	0.25	0	0	0	0	
B				Aldehydes								
1	23.2	1504	1502**	Benzaldehyde	0.07	0	0	0.8	0	0	0	
2	33.5	1836	1410***	Dodecanal	6.63	0.67	0.18	0.4	0	1.22	0.2	
3	40.4	2103		Octadecanal	4.86	1.25	0.76	0.53	1.8	7.53	0.6	
C				Furan Derivatives								
1	21.6	1456	1474****	2-furancarboxaldehyde	0.71	0	0.19	0.96	0.9	0.68	1.2	
2	27.97	1652	1657*****	2-furanmethanol	0.21	0	0.47	0.99	0	0.69	2.3	
D				Ester Derivatives								
1	4.25	901	888*****	Ethyl Acetate	1.16	1.3	6.47	5.65	3.3	1.22	4.7	
2	58.1	2200	1356*	Butyl octyl ester	0	0	0	0	0	0	0.6	

^a L= Yogurt with single *L. bulgaricus* culture; LB= Yogurt with *L. bulgaricus* and *B. longum* cultures; S= Yogurt with single *S. thermophilus* culture; SB= Yogurt with *S. thermophilus*+*B. longum* cultures; LS= Yogurt with *L. bulgaricus*+*S. thermophilus* cultures; LSB= Yogurt with *L. bulgaricus*+*S. thermophilus*+*B. longum* cultures; B= Yogurt with single *B. longum* culture; RT= Retention Time; LRI= Linear Retention Index; Ref= Reference. *Tian *et al.*, 2019; **Sakho *et al.*, 1985; *** Zhang *et al.*, 2020; ****Wong and Tie, 1993; *****Shiratsuci *et al.*, 1994.

The results of yogurt aroma analysis conducted using GC-MS showed the presence of dodecanal, benzaldehyde, and octadecanal aldehyde groups. Both octadecanal and dodecanal were found to be the most dominant. Octadecanal was present in all treatments, while dodecanal was not detected in SB2 and S. These compounds have no significant effect on aroma of yogurt, and aldehydes are known for the stimulating and fruity aroma (Reineccius, 2006).

Furan derivatives found in yogurt are chemical compounds with furan rings in the structure. These have been detected in various foods, including coffee, canned/bottled product, cereal-based product, and thermally processed foods such as rye bread. However, the presence of furan derivatives in yogurt remains unclear. More investigations or special studies are needed to determine its presence in this food product (Arisseto, 2016; Tameko *et al.*, 2017). The heating process particularly carried out during milk pasteurization before

fermentation may lead to the formation of furan derivatives in yogurt (Batool *et al.*, 2023). Furan derivatives can be formed during milk heating, specifically in the context of Ultra High Temperature (UHT) processing for yogurt production.

In LS, 2-furancarboxaldehyde increased and 2-furanmethanol decreased, which was a trend similarly observed in all yogurt treatments. These two compounds are primarily produced in yogurt with treatment B. The relationship between bacterial activity in producing 2-furancarboxaldehyde and 2-furanmethanol is not yet understood, as both compounds are formed during the heating process. According to Guo *et al.* (2019) and Asaduzzaman *et al.* (2021), the heating process of milk in yogurt production significantly affects furan formation. Furan has the potential to be a carcinogenic compound that can form during the thermal processing of heated food.

Ester derivatives are chemical compounds related to esters, which are organic groups



formed from the reaction between carboxylic acids and alcohols. The structures and properties of the derivatives can vary depending on the type of carboxylic acids and alcohol used in the synthesis. Moreover, esters are formed during the esterification process through the reaction of lactic acids with other acids in milk. These compounds play a role in giving yogurt a distinctive taste and aroma, although the contribution is not very significant. Farooq and Haque (1992) stated that sugar esters could improve the general quality of yogurt, enhancing the texture, taste, and mouthfeel. Vásquez-Trespalacios and Romero-Palacio (2014) found that yogurt drinks with added plant stanol esters significantly reduced total and LDL cholesterol.

Ethyl acetate, such as esters, is formed through an esterification reaction between carboxylic acids and alcohol, probably due to the better solubility of ethyl acetate compared to butyl octyl ester. Furthermore, it is suspected to be more volatile, leading to the dominance during analysis with GC-MS. Cheng (2010) described aroma of ethyl acetate as solvent-like and fruity, resembling pineapple. Ni *et al.* (2022) found this aroma to induce cytotoxicity in breast cancer without affecting normal cells.

The results will provide valuable insights into the production of yogurt with a distinct aroma, enhancing product competitiveness in a rapidly growing market. Additionally, this study can contribute to advancing knowledge on fermented milk product and the practical applications in the food and beverage industry.

CONCLUSIONS

In conclusion, yogurt is a fermented dairy product prioritized for the nutritional benefits and unique aroma, commonly influenced by the ingredients and fermentation process. The production of yogurt from cow milk often uses live cultures such as *L. bulgaricus* and *S. thermophilus*. Therefore, this study explores the effect of different probiotic bacteria

combinations on aroma of yogurt by identifying components such as acids, alcohols, ketones, and aldehydes. Octanoic, decanoic, and dodecanoic acids are the most prominent among the 10 types of acids found, contributing to aroma complexity. Aldehydes and furan derivatives formed during heating are not highly significant. The results show the importance of selecting specific probiotic combinations to achieve the desired aroma profile, which helps to improve yogurt marketability.

REFERENCES

1. Adams, R. P. 2007. *Identification of Essential Oil Components by Gas Chromatography/ Mass Spectrometry*. 4th Edition, Allured Publication, Carol Stream, Illinois, USA.
2. Adrianto, R., Wiraputra, D., Daniro Jyoti, M. and Zulaika, A. 2020. Total Bacteria of Lactic Acid, Total Acid, pH Value, Syneresis, Total Dissolved Solids and Organoleptic Properties of Yoghurt Back Slooping Method. *J. Agritechno*, **13(2)**: 105–111.
3. Aktar, T. 2022. Physicochemical and Sensory Characterization of Different Yoghurt Production Methods. *Int. Dairy J.*, **125**: 105245–105245.
4. Arisseto, A. P. 2016. Furan in Processed Foods. In: "*Food Hygiene and Toxicology in Ready-to-Eat Foods*". Academic Press, PP. 383–396.
5. Arslaner, A. 2020. The Effects of Adding Garlic (*Allium sativum* L.) on the Volatile Composition and Quality Properties of Yogurt. *Food Sci. Technol. Int.* **40**: 582–591.
6. Asaduzzaman, M., Mahomud, M. S. and Haque, M. E. 2021. Heat-Induced Interaction of Milk Proteins: Impact on Yoghurt Structure. *Int. J. Food Sci.*, **Volume 2021, Article ID 5569917, 10 PP.**
7. Barros, R. F., Cutrim, C. S., Da Costa, M. P., Junior, C. A. C. and Cortez, M. A. S. 2019. Lactose Hydrolysis and Organic Acids Production in Yogurt Prepared with Different Onset Temperatures of Enzymatic Action and Fermentation. *Ciênc. Anim. Bras.*, **20**: 1–10.
8. Batool, Z., Chen, J. H., Liu, B., Chen, F. and Wang, M. 2023. Review on Furan as a Food

- Processing Contaminant: Identifying Research Progress and Technical Challenges for Future Research. *J. Agric. Food Chem.*, **71(13)**: 5093–5106.
9. Cheng, H. 2010. Volatile Flavor Compounds in Yogurt: A Review. *Crit. Rev. Food Sci. Nutr.*, **50(10)**: 938–950.
 10. Chen, C., Zhao, S., Hao, G., Yua, H., Tian, H. and Zhao, G. 2017. Role of lactic acid bacteria on the yogurt flavour: A review. *International Journal of Food Properties* 2017, Vol. 20, No. S1, S316–S330 <https://doi.org/10.1080/10942912.2017.1295988>
 11. Eker, T. and T. Cabaroğlu. 2020. The Impact Factors of Yogurt Matrix on in Vivo Aroma Release during Consumption of Yogurt. *GIDA*, **45(1)**: 50–60.
 12. Elbarbary, H. A. 2014. Using of Some *Bifidobacteria* Species as Biopreservative Cultures in Some Dairy Products. *Assiut Vet. Med. J.*, **60(143)**: 54–60.
 13. Fadhlorrohan, I., Sumarmono, J., Tianling, M., Prasetya, R., Safitri, A., Kafa, U. A. and Setyawardani, T. 2023. Physical and Chemical Properties of Cow's Milk Yogurt Added Whey Protein Concentrate (WPC). *Proceeding ICMA-SURE*, **2**: 109–113.
 14. Farooq, K. and Haque, Z. U. 1992. Effect of Sugar Esters on the Textural Properties of Nonfat Low Calorie Yogurt. *J. Dairy Sci.*, **75(10)**: 2676–2680.
 15. Guo, J., Zhao, R., Li, J., Wu, D., Yang, Q., Zhang, Y. and Wang, S. 2019. Furan Formation from Ingredient Interactions and Furan Mitigation by Sugar Alcohols and Antioxidants of Bamboo Leaves in Milk Beverage Model Systems. *J. Sci. Food Agric.*, **99(11)**: 4993–4999.
 16. He, B. L., Xiong, Y., Hu, T. G., Zong, M. H. and Wu, H. 2023. *Bifidobacterium* spp. as Functional Foods: A Review of Current Status, Challenges, and Strategies. *Crit. Rev. Food Sci. Nutr.*, **63**: 8048–8065.
 17. Hussain, S. A., Panjagari, N. R., Singh, R. R. B. and Patil, G. R. 2010. Production and Function of Dealcohol Yogurt Beverage. *China Dairy Industry*, **38(1)**: 26–28.
 18. Jegal, S., Heacock, H., Trmcic, A. and Shaw, F. 2019. The Effect of Probiotics as a Starter Culture for Producing Yogurt. *BCIT Environmental Public Health Journal*, 48–99. <https://journals.bcit.ca/index.php/ehj/article/view/41/30>
 19. Kamara, D. S., Rachman, S. D., Pasisca, R. W., Djajasoepana, S., Suprijana, O., Idar, I. and Ishmayana, S. 2016. Preparation and Antibacterial Activity of Yogurt Fermented by Three Bacteria [Pembuatan dan Aktivitas Antibakteri Yogurt Hasil Fermentasi Tiga Bakteri (*Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*)]. *Al-Kimia*, **4(2)**: 121–131.
 20. Krastanov., A, Georgiev, M., Slavchev, A., Blazheva, D., Goranov, B. and Ibrahim, S. A. 2023. Design and Volatile Compound Profiling of Starter Cultures for Yogurt Preparation. *Foods*, **12(2)**: 1–18.
 21. Latif, A., Shehzad, A., Niazi, S., Zahid, A., Ashraf, W., Iqbal, M. W., Rehman, A., Riaz, T., Aadil, R. M., Khan, I. M., Özogul, F., Rocha, J M, Esatbeyoglu, T. and Korma, S. A. 2023. Probiotics: Mechanism of Action, Health Benefits and Their Application in Food Industries. *Front. Microbiol.*, **14**: 1–15.
 22. Li, H., Gao, J., Chen, W., Qian, C., Wang, Y., Wang, J. and Chen, L. 2022. Lactic Acid Bacteria Isolated from Kazakh Traditional Fermented Milk Products Affect the Fermentation Characteristics and Sensory Qualities of Yogurt. *Food Sci. Nutr.*, **10(5)**: 1451–1460.
 23. Liu, A., Liu, Q., Bu, Y., Hao, H., Liu, T., Gong, P., Zhang, L., Chen, C., Tian, H. and Yi, H. 2022. Aroma Classification and Characterization of *Lactobacillus delbrueckii* subsp. *bulgaricus* Fermented Milk. *Food Chem.* **X(15)**: 100385–100385.
 24. Liu, C., Yang, P., Wang, H. and Song, H. 2022. Identification of Odor Compounds and Odor-Active Compounds of Yogurt Using DHS, SPME, SAFE, and SBSE/GC-O-MS. *LWT*, **154**: 1–10.
 25. Li, L. Q., Chen, X., Zhu, J., Zhang, S., Chen, S. Q. and Liu, X. 2023. Advances and Challenges in Interaction between Heteroglycans and *Bifidobacterium*: Utilization Strategies, Intestinal Health and Future Perspectives. *Trends Food Sci. Technol.*, **134**: 112–122.
 26. Li, L., Zhang, C., Liu, B., Tian, F., Yu, L., Chen, W. and Zhai, Q. 2024. Factor Involved in the Abundant Dominance of *Bifidobacterium longum* within the Genus in the Human Gut. *Food Biosci.*, **61**: 1–13.
 27. Nadal, I., Rico, J., Pérez-Martínez, G., Yebra, M. J., & Monedero, V. 2009.



- Diacetyl and Acetoin Production from Whey Permeate Using Engineered *Lactobacillus casei*. *J. Ind. Microbiol. Biotechnol.*, **36(9)**: 1233–1237.
28. Nair, S. and Peerkhan, N. 2022. Formulation of Millet Milk and Herb Extract Enriched Yogurt and to Assess Its Nutritional Characteristics : Life Sciences-Nutrition and Dietetics. *Int. J. Life Sci. Pharma Res.*, **13(1)**: L151-L163.
29. Ni, B., Li, W., Ifrah, K., Du, B., Xu, Y., Zhang, C. and Li, X. 2022. Dynamic Transcriptome Analysis Reveals Transcription Factors Involved in the Synthesis of Ethyl Acetate in Aroma-Producing Yeast. *Genes*, **13(12)**: 2341–2341.
30. Nurhayati, Jayus, J., Rini, A. W. E., Sugiharto, B. and Rahmanto, D. E. 2022. Isolation and Identification of Alcohol-Tolerant Yeast from Molasses (Isolasi dan Identifikasi Khamir Toleran Alkohol dari Molase). *Biota: J. Ilmiah Ilmu Ilmu Hayati*, **7(1)**: 1–10.
31. Reineccius, G. 2006. *Flavor Chemistry and Technology*. Taylor and Francis, Boca Raton, FL.
32. Sakho, M., Crouzet, J. and Seck, S., 1985. Volatile Components of African Mango. *J. Food Sci.*, **50(2)**: 548-550.
33. Sharma, M., Wasan, A. and Sharma, R. K. 2021. Recent Developments in Probiotics: An Emphasis on *Bifidobacterium*. *Food Biosci.* **41**: 1-10.
34. Shiratsuchi, H., Shimoda, M., Imayoshi, K., Noda, K. and Osajima, Y. 1994. Volatile Flavor Compounds in Spray-Dried Skim Milk Powder. *J. Agric. Food Chem.*, **42**: 984-988.
35. Siddiqi, M., Taraah, A., Chen, Z. H. and LaPointe, G. 2024. Phenotypic Differentiation of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* Isolates Found in Yogurt Starter Cultures. *Fermentation*, **10(12)**: 1-13.
36. Swelam, S., Rashed, M., & khames, E. 2019. Properties of Non-Fat Yoghurt as Influenced by The Incubation Temperature of Exopolysaccharide Producing Culture. *J. Food Dairy Sci.*, **10(12)**: 447–452.
37. Tameko, J. E. M., Chouna, J. R., Nkeng-Efouet-Alango, P., Tapondjou, L. A. and Sewald, N. 2017. Furan derivatives from *Lannea kerstingii*. *Phytochem. Lett.*, **20**: 282–284.
38. Tian, H., Shi, Y., Zhang, Y., Yu, H., Mu, H. and Chen, C. 2019. Screening of Aroma-Producing Lactic Acid Bacteria and Their Application in Improving the Aromatic Profile of Yogurt. *J. Food Biochem.*, **43(10)**: e12837.
39. Tian, H., Yu, B., Yu, H. and Chen, C. 2020. Evaluation of the Synergistic Olfactory Effects of Diacetyl, Acetaldehyde, and Acetoin in a Yogurt Matrix Using Odor Threshold, Aroma Intensity, and Electronic Nose Analyses. *J. Dairy Sci.*, **103(9)**: 7957–7967.
40. Trentin, M. M., Becker, A. F., Hanauer, D., Bianchi, A., Borges, L. and Schogor, A. L. B. 2022. Development of Yogurts with Mixture of Sheep and Bovine Milk: Effect on Chemical Physical Characteristics, Protein Profile and Antioxidant Activity. *Res. Soc. Dev.*, **11(16)**: 1-18.
41. Vásquez-Trespalcios, E. M. and Romero-Palacio, J. 2014. Efficacy of Yogurt Drink with Added Plant Stanol Esters (Benecol® , Colanta) in Reducing Total and LDL Cholesterol in Subjects with Moderate Hypercholesterolemia: A Randomized Placebo-Controlled Crossover Trial NCT01461798. *Lipids Health Dis.*, **13(1)**: 1-7.
42. Wihansah, R. R. S., Pazra, D. F., Wahyuningsih and Handayani, K. S. 2022. Assessment of the Antidiabetic Activity and Characteristics of Cow's Milk Yogurt Enhanced with Herbs Extracts. *IOP Conf. Ser.: Earth Environ. Sci.*, **1020(1)**: 1-10
43. Wong, K. C. and Tie, D. Y. 1995. Volatile Constituent of Durians (*Durio ziberthimus* Murr). *Flav. Fragr. J.*, **10(2)**: 79-83.
44. Yu, H. S., Lee, N. K., Jeon, H. L., Eom, S. J., Yoo, M. Y., Lim, S. D. and Paik, H. D. 2016. Benzoic Acid Production with Respect to Starter Culture and Incubation Temperature during Yogurt Fermentation using Response Surface Methodology. *Korean J. Food Sci. Anim. Resour.*, **36(3)**: 427–434.
45. Yüksel, A. K. and Bakırcı, İ. 2015. An Investigation of the Volatile Compound Profiles of Probiotic Yogurts Produced Using Different Inulin and Demineralised Whey Powder Combinations. *Food Sci. Biotechnol.*, **24(3)**: 807–816.

46. Zhang, L., Mi, S., Liu, R., Sang, Y. and Wang, X. 2020. Evaluation of Volatile Compounds during the Fermentation Process of Yogurts by *Streptococcus thermophilus* Based on Odor Activity Value and Heat Map Analysis. *Int. J. Anal. Chem.* **Volume 2020, Article ID 3242854, 10 PP.**
47. Zhang, S. and Zhang, L. 2012. Effect of Exopolysaccharide Producing Lactic Acid Bacterial on the Gelation and Texture Properties of Yogurt. *Adv. Mater. Res.*, **430-432**: 890-893.
48. Zhao, M., Li, H., Zhang, D., Li, J., Wen, R., Ma, H., Zou, T., Hou, Y. and Song, H. 2023. Variation of Aroma Components of Pasteurized Yogurt with Different Process Combination before and after Aging by DHS/GC-O-MS. *Molecules*, **28(4)**: 1975-1975.
49. Znamenska, T and O. Vorobiova. 2024. Functions and Individual Mechanisms of Influence of Bifidobacteria on The State of Health of Infants (Literary Review). *Neonatal. Surg. Perinat. Med.*, **14(4)(54)**: 191-195.

بوی (عطر) ماست از شیر گاو با ترکیبی از چندین باکتری پروبیوتیک

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چکیده

این پژوهش با هدف بررسی تأثیر باکتری‌های پروبیوتیک (Probiotic Bacteria) مختلف بر بوی (عطر) ماست از طریق یک فرآیند آنکوباسیون منحصر به فرد انجام شد. باکتری‌های پروبیوتیک مورد بررسی شامل لاکتوباسیلوس (*Lactobacillus*)، استرپتوکوک (*Streptococcus*) و بیفیدوباکتریوم (*Bifidobacterium*) بودند. علاوه بر این، فرآیند تولید ماست شامل استریل کردن شیر بدون چربی، افزودن کشت‌های باکتریایی و گرمخانه‌گذاری در دماهای خاص بود. اجزای بوی (عطر) ماست با استفاده از کروماتوگرافی گازی و طیف‌سنجی جرمی مورد تجزیه و تحلیل قرار گرفتند. نتایج نشان داد که ماست حاوی ترکیبات معطر مختلفی است که در چهار دسته اسیدها، الکل‌ها، کتون‌ها و آلدئیدها طبقه‌بندی می‌شوند. اجزای اسیدی غالب شامل استیک اسید، اکتانویک اسید، دکانویک اسید و دودکانویک اسید بودند. در میان اجزا، الکل، با وجود مقادیر کم، برعطر تأثیر گذاشت. کتون‌هایی مانند استوئین و دی‌استیل به همراه آلدئیدهایی از جمله اکتادکانال و دودکانال در ماست شناسایی شدند. این پژوهش، داده‌های ارزشمندی در مورد تأثیر باکتری‌های پروبیوتیک بر پروفایل بو و عطر ماست ارائه داد و به تولیدکنندگان در اصلاح فرآیندهای تولید برای تهیه محصولی جذاب برای مصرف‌کنندگان کمک کرد.