

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

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4 **Abstract**

5 Yogurt is a widely consumed fermented product celebrated for nutritional benefits and
6 distinctive aroma, influenced by factors such as the type of probiotic bacteria, incubation
7 methods, and the used milk type. Common bacteria in yogurt production include *Lactobacillus*
8 *bulgaricus* and *Streptococcus thermophilus*. Aroma is crucial for consumers' acceptance and
9 varies according to the compounds formed during fermentation. Therefore, this study aimed to
10 investigate the effect of different probiotic bacteria on aroma of yogurt through a unique
11 incubation process. Probiotic bacteria examined were Lactobacillus, Streptococcus, and
12 Bifidobacterium. Additionally, yogurt production process included sterilizing skim milk,
13 introducing bacterial cultures, and incubating at specific temperatures. Aroma components of
14 yogurt were analyzed using Gas Chromatography and Mass Spectrometry. The result showed
15 that yogurt comprised various aroma compounds classified into four categories, namely acids,
16 alcohols, ketones, and aldehydes. The dominant acid components found were acetic, octanoic,
17 decanoic, and dodecanoic acids. Among the components, alcohol affected aroma despite being
18 present in minimal quantities. Ketones such as acetoin and diacetyl were identified along with
19 aldehydes including octadecanal and dodecanal in yogurt. This study provided valuable insights
20 into the effect of probiotic bacteria on aroma profile of yogurt, assisting manufacturers in
21 refining production processes to prepare product appealing to consumers.

22 **Keywords:** aroma compounds, Gas Chromatography, Mass Spectrometry, probiotic bacteria,
23 yogurt production.

24
25 **Introduction**

26 Yogurt is a popular fermented milk product known for the nutritional value and unique aroma,
27 which varies depending on the used ingredients. The major ingredient in yogurt is primarily
28 cow milk, and live cultures such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus*
29 are commonly used during the production. Additional flavorings or sweeteners may be added
30 to customize the taste of yogurt (Nair & Peerkhan, 2022; Trentin et al., 2022; Wihansah et al.,
31 2022; Fadhlurrohman et al., 2023).

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32 Aroma of yoghurt plays a crucial role in the acceptance by consumers and this varies
33 significantly based on factors such as the type of probiotic bacteria used in fermentation, milk
34 type, and the incubation methods applied. According to Chen (2017), yogurt production is a
35 complex process that generates a distinctive aroma. Zhao et al. (2023) reported that factors such
36 as the production process, extraction method, starter culture concentration, and incubation time
37 could further affect aroma.

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

41 A key factor in shaping the preferences of consumers for yogurt product is aroma, which the
42 perceptions greatly determine purchase decisions and continual product enjoyment. This shows
43 the need to comprehend the various aroma components of yogurt as well as the effect on
44 consumers' preferences in the food and beverage industry. Aroma is affected by several factors,
45 including the type of bacteria in the starter culture, the processing method, the source of milk
46 and chemicals, and any additional ingredients used. According to Eker et al. (2020), aroma is
47 essential in shaping the preferences of consumers and the general sensory experience of yogurt.

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

53 Probiotic bacteria used in this study were *Lactobacillus*, *Streptococcus*, and *Bifidobacterium*
54 which provide health benefits and produce various aroma components. The combination of the
55 three bacteria is expected to help with the production of high-quality yogurt due to containing
56 a harmonious blend. According to Siddiqi et al. (2024), *L. bulgaricus* and *S. thermophilus*
57 bacteria contribute to a decrease in pH, which is important for the texture and flavor of yogurt.
58 Bifidobacteria is among the dominant colonies in the human gut, specifically in infants (He et
59 al., 2023; Znamenska and Vorobiova, 2024).

60 Kamara et al. (2016) reported that yogurt
61 fermented with mixed cultures of *L. bulgaricus*, *S. thermophilus*, and *L. acidophilus* showed
62 antibacterial activity against *E. coli* and *Bacillus subtilis*. A study by Elbarbary (2014) found
63 that the inclusion of bifidobacteria spp. in stored yogurt inhibited the growth of *E. coli* and
64 *Staphylococcus aureus*.

65 During yogurt fermentation, probiotic bacteria used are essential in developing the unique
66 aroma of the finished product and various strains can generate different aroma compounds.

66 Therefore, selecting the appropriate probiotic combination is crucial in achieving the desired
67 aroma.

68 Several previous investigations focused on different culture treatments and temperature
69 variations (Yu et al., 2016; Swelam et al., 2019; Jegal et al., 2019; Adrianto et al., 2020).

70 However, a unique method was used in this study where *Bifidobacteria* was incubated with *L.*
71 *bulgaricus* and *S. thermophilus* cultures, as well as in a separate manner.

72 This study aimed to examine the effect of various probiotic bacteria on yogurt aroma.

73 Understanding the effect of microbial factors can help yogurt producers enhance manufacturing
74 processes to prepare product with an appealing aroma that resonates with consumers. Aroma
75 profile in this study is determined by the presence of volatile components.

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

80

81 **Material and Method**

82 **Material and Equipment**

83 Commercial powdered skim milk is used in yogurt production, along with bacteria strains *L.*
84 *bulgaricus* 18 Visbyvac Serie 50 No. 700398 (*L. bulgaricus*), *S. thermophilus* A Vysbivac No.
85 640638 (*S. thermophilus*), and *Bifidobacterium longum* BF1 (*B. longum*). Additionally, the
86 laboratory equipment used were a rotary evaporator, Gas Chromatograph (Shimadzu 17 A), and
87 Mass Spectrometer (Shimadzu QP 5000), as well as Erlenmeyer tubes and other necessary glass
88 tools.

89

90 **Yogurt Preparation and Production**

91 Skim milk powder was dissolved in distilled water until a specific gravity of 1.027 equivalent
92 to fresh milk was attained. The mixture was sterilized at 121°C for 15 minutes in an autoclave.

93 After cooling to 42°C, a commercial culture of *L. bulgaricus* / *S. thermophilus* was added at a
94 concentration of 2% of the sterilized milk volume. The resulting mixture was incubated at 37°C

95 for 20 hours, then cooled to 37°C before adding *B. longum* to the milk. *B. longum* was selected
96 over more commonly used probiotic due to the superior adaptability and health benefits

97 provided in the human gut (Sharma et al., 2022; Li et al., 2023; Li et al., 2024).

98

99

100

101 **Preparing Samples for the Analysis of Aromatic Compounds**

102 The separation of volatile aroma components is conducted using the Likens-Nickerson
 103 equipment and SDE (Simultaneous Distillation Extraction) method. A Gas Chromatograph was
 104 used in combination with a Mass Spectrometer (GC-MS) to analyze aroma components
 105 following the method described by Shiratsuchi et al. (1994). A solution of 300 g yogurt
 106 dissolved in 900 ml distilled water was used to separate aroma components, with Polyethylene
 107 glycol 4000 added to prevent foaming. Subsequently, extraction and distillation were carried
 108 out simultaneously for 1 hour using 30 ml of diethyl ether as the solvent. Anhydrous sodium
 109 sulfate was added to bind water, and the solution was concentrated with a rotary evaporator.
 110 Nitrogen gas was used to obtain the distillate, which was injected into GC-MS apparatus. This
 111 process was duplicated and analyzed under the following conditions presented in Table 1.

112 **Table 1.** Condition of GC-MS Instrument Used.

The 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-splttless; 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

113
 114 Yogurt was produced in this study using the incubation method with a freeze-dried *B. longum*
 115 bacterial culture. The incubation process of the culture was carried out for 20 hours at 37°C.
 116 Seven combination treatments were performed using probiotic bacteria and the incubation
 117 method during yogurt production. These include L = single culture of *L. bulgaricus*, 2) LB1 =
 118 *L. bulgaricus* + *B. longum* incubated, 3) S = single culture of *S. thermophilus*, 4) SB1 = *S.*
 119 *thermophilus* + *B. longum* incubated, 5) LS = *L. bulgaricus* + *S. thermophilus*, 6) LSB1 = *L.*
 120 *bulgaricus* + *S. thermophilus* + *B. longum* incubated, and 7). B = single culture of *B. longum*,
 121 from which the results are reported descriptively.

122

123 **Results and Discussion**

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

127
128 **Group of Acids**

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

133 **Table 2.** Group of Acids.

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

134 Note: L= Yogurt with single *L. bulgaricus* culture; LB= Yogurt with *L. bulgaricus* and *B. longum* cultures; S= Yogurt with
135 single *S. thermophilus* culture; SB= Yogurt with *S. thermophilus* + *B. longum* cultures; LS= Yogurt with *L. bulgaricus* + *S.*
136 *thermophilus* cultures; LSB= Yogurt with *L. bulgaricus* + *S. thermophilus* + *B. longum* cultures; B= Yogurt with single *B.*
137 *longum* culture; RT= Retention Time; LRI= Linear Retention Index; Ref= Reference.

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

139
140 Yogurt is a dairy product containing various acids, such as lactic acids essential for the taste
141 and texture. During fermentation process, lactic acid bacteria (LAB), such as *L. bulgaricus* and
142 *S. thermophilus*, consume the lactose in milk and convert it into lactic acids. The tangy flavor
143 of yogurt originates from lactic acids which also helps prevent the growth of harmful bacteria
144 and contributes to the thick product consistency. The performance of LAB starters directly
145 affects the quality of yogurt, including the texture and gel properties (Zhang & Zhang, 2012).
146 LAB from traditionally prepared dairy product, such as *Lactobacillus plantarum*, was found to
147 enhance the condensed and textural integrity of yogurt (Li et al., 2022).

148 Acids are long carbon chain fat molecules with a carboxylic acid group at the terminal portion.
149 The milk used in yogurt production contains both saturated and unsaturated acids, which
150 contribute to the characteristic taste and aroma. Additionally, acids play a role in the thickness

151 and texture of yogurt. Some acids, such as butyric (butanoic) and caproic (octanoic) acids, can
152 impart a buttery taste to yogurt, while others, including acetic and hexanoic acids, contribute to
153 a soft and fruity aroma. According to Zhao et al. (2023) and Tian et al. (2019), aroma is
154 influenced by various acids, including butyric, caproic, acetic, and hexanoic acids, which
155 collectively contribute to the general flavor profile and sensory characteristics of yogurt.

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

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

164 Yogurt aroma analysis in this study found that acetic acids were the first to appear, therefore,
165 considered as the most volatile component (Zhao et al., 2023) detected using GC-MS (Arslaner,
166 2020; Yüksel & Bakırcı, 2015; Cheng, 2010). According to Cheng (2010), acetic acids
167 contribute to the vinegar-like, pungent, and acidic aroma of yogurt.

168 Table 2 shows that certain yogurt using different cultures and combinations (L, LB, B) produce
169 10 types of volatile acid components. Some treatments, including S, SB, LS, and LSB,
170 generated fewer than 10 types of volatile acids. In this study, yogurt with a single culture of *L.*
171 *bulgaricus* produced 11 acid group compounds. Liu et al. (2022) identified 12 aroma-active
172 compounds in yogurt, including butanoic, acetic, and hexanoic acids, as the main aroma-active
173 compounds in fermented milk produced by *L. bulgaricus*. The results showed that yogurt
174 produced with a single culture of *L. bulgaricus* obtained these compounds. Treatments with a
175 single culture of *S. thermophilus* (S), as well as incubation with *L. bulgaricus* (LS) and
176 Bifidobacteria (SB), did not produce benzoic acid. According to Yu et al. (2016), benzoic acid
177 production has been studied for various starter cultures and incubation temperatures without a
178 report on the optimization process. The use of a single culture of Bifidobacteria in preparing
179 yogurt produces 10 types of components, among which the most prominent are octanoic,
180 decanoic, and dodecanoic acids. Tian et al. (2019) found that both octanoic and decanoic acids
181 contributed to the distinctive taste of cheese, while octanoic acids produced a light creamy taste
182 in yogurt.

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

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

190

191 Group of Alcohol

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

197

Table 3. Group of Alcohol.

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

198 Note: L= Yogurt with single *L. bulgaricus* culture; LB= Yogurt with *L. bulgaricus* and *B. longum* cultures; S= Yogurt with
199 single *S. thermophilus* culture; SB= Yogurt with *S. thermophilus* + *B. longum* cultures; LS= Yogurt with *L. bulgaricus*+*S.*
200 *thermophilus* cultures; LSB= Yogurt with *L. bulgaricus* + *S. thermophilus*+*B. longum* cultures; B= Yogurt with single *B.*
201 *longum* culture; RT= Retention Time; LRI= Linear Retention Index; Ref= Reference.

202 *= Adams, 2007.

203

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

211

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

213 Ketones are a type of compound included in the carbonyl group and not the major product
214 formed during yogurt fermentation process. Table 4 shows that acetoin and diacetyl are the
215 most abundant compounds produced in ketone group.

216

217 **Table 4.** Groups of Ketone Compounds, Aldehydes, Furan Derivatives, and Ester Derivatives.

No.	RT	LRI	LRI (Ref)	Compound Type	Treatments						
					L	LB	S	SB	LS	LSB	B
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

218 Note: L = Yogurt with single *L. bulgaricus* culture; LB= Yogurt with *L. bulgaricus* and *B. longum* cultures; S= Yogurt with
219 single *S. thermophilus* culture; SB= Yogurt with *S. thermophilus* + *B. longum* cultures; LS= Yogurt with *L. bulgaricus* + *S.*
220 *thermophilus* cultures; LSB= Yogurt with *L. bulgaricus* + *S. thermophilus* + *B. longum* cultures; B= Yogurt with single *B.*
221 *longum* culture; RT= Retention Time; LRI= Linear Retention Index

222 Ref= Reference.

223 *Tian et al., 2019; **Sakho et al., 1985; *** Zhang et al., 2020; ****Wong & Tie, 1993

224 *****Shiratsuci et al., 1994.

225
226 Acetoin and diacetyl are both ketone compounds commonly used in foods and drinks to enhance
227 specific flavors and aroma. For example, acetoin can impart a buttery taste to yogurt, while
228 diacetyl provides an acidic or buttery flavor. This study showed that all treated yogurt produced
229 acetoin, and diacetyl was only generated by six (L, S, SB, LS, LSB, B) out of the seven
230 treatments. These two are the primary aroma compounds in yogurt playing a significant role in
231 determining the general aroma and taste (Tian et al., 2020). Acetoin is produced by *L.*
232 *acidophilus* during fermentation, while diacetyl is formed through the lipolysis of milk fat as
233 well as the microbiological transformation of lactose and citrate (Cheng, 2010). The odor
234 threshold for acetoin and diacetyl in yogurt is higher than in water (Nadal et al., 2009). Acetoin
235 is commonly found in food fermentation product due to being a metabolic byproduct of
236 microorganisms such as lactic acid bacteria. In yogurt, acetoin is typically present in higher
237 concentrations than other ketone compounds, leading to easy detection with GC-MS and a more
238 prominent appearance. This is attributed to acetoin being a compound produced by the
239 metabolism of LAB commonly used in the production of yogurt.

240 Aldehydes are included in the group of carbonyl compounds, along with ketones. The main
241 difference between ketones and aldehydes depends on the atoms attached to the carbonyl group.
242 In ketones, the carbonyl group is bonded to two carbon atoms, while in aldehydes, it is bonded
243 to one carbon and a hydrogen atom. Aldehydes are present in very small amounts as by-product

244 during fermentation process but not considered a major component or main characteristic of
245 yogurt.

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

251 Furan derivatives found in yogurt are chemical compounds with furan rings in the structure.
252 These have been detected in various foods, including coffee, canned/bottled product, cereal-
253 based product, and thermally processed foods such as rye bread. However, the presence of furan
254 derivatives in yogurt remains unclear. More investigations or special studies are needed to
255 determine the presence in this food product (Arisseto, 2016; Tameko et al., 2017). The heating
256 process particularly carried out during milk pasteurization before fermentation may lead to the
257 formation of furan derivatives in yogurt (Batoool et al., 2023). Furan derivatives can be formed
258 during milk heating, specifically in the context of UHT (Ultra High Temperature) processing
259 for yogurt production.

260 In LS, 2-furancarboxaldehyde increased and 2-furanmethanol decreased, which was a trend
261 similarly observed in all yogurt treatments. These two compounds are primarily produced in
262 yogurt with treatment B. The relationship between bacterial activity in producing 2-
263 furancarboxaldehyde and 2-furanmethanol is not yet understood, as both compounds are
264 formed during the heating process. According to Guo et al. (2019) and Asaduzzaman et al.
265 (2021), the heating process of milk in yogurt production significantly affects furan formation.
266 Furan has the potential to be a carcinogenic compound that can form during the thermal
267 processing of heated food.

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

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

283

284 Conclusions

285 In conclusion, yogurt is a fermented dairy product prioritized for the nutritional benefits and
286 unique aroma, commonly influenced by the ingredients and fermentation process. The
287 production of yogurt from cow milk often uses live cultures such as *L. bulgaricus* and *S.*
288 *thermophilus*. Therefore, this study explores the effect of different probiotic bacteria
289 combinations on aroma of yogurt by identifying components such as acids, alcohols, ketones,
290 and aldehydes. Octanoic, decanoic, and dodecanoic acids are the most prominent among the 10
291 types of acids found, contributing to aroma complexity. Aldehydes and furan derivatives
292 formed during heating are not highly significant. The results show the importance of selecting
293 specific probiotic combinations to achieve the desired aroma profile, which helps to improve
294 yogurt marketability.

295

296 Reference

- 297 1. Adams, R. P. 2007. Identification of essential oil components by gas chromatography/
298 mass spectrometry, 4th Edition. Allured Publ. Carol Stream, Illinois, USA.
- 299 2. Adrianto, R., Wiraputra, D., Daniro Jyoti, M., & Zulaika, A. 2020. Total Bacteria of
300 Lactic Acid, Total Acid, pH Value, Syneresis, Total Dissolved Solids and Organoleptic
301 Properties of Yoghurt Back Slooping Method. *Jurnal Agritechno*, 13(2), 105–111.
302 <https://doi.org/10.20956/AT.V13I2.358>
- 303 3. Aktar, T. 2022. Physicochemical and sensory characterization of different yoghurt
304 production methods. *International Dairy Journal*, 125, 105245–105245.
305 <https://doi.org/10.1016/j.idairyj.2021.105245>
- 306 4. Arisseto, A. P. 2016. Furan in processed foods. *Food Hygiene and Toxicology in Ready-*
307 *to-Eat Foods*, 383–396. <https://doi.org/10.1016/B978-0-12-801916-0.00021-2>
- 308 5. Arslaner, A. 2020. The effects of adding garlic (*Allium sativum* L.) on the volatile
309 composition and quality properties of yogurt. *Food Science and Technology International*, 40,
310 582–591. <https://doi.org/10.1590/FST.31019>

- 311 6. Asaduzzaman, M., Mahomud, M. S., & Haque, M. E. 2021. Heat-Induced Interaction
312 of Milk Proteins: Impact on Yoghurt Structure. *International Journal of Food Science*, 2021,
313 5569917. <https://doi.org/10.1155/2021/5569917>
- 314 7. Barros, R. F., Cutrim, C. S., Da Costa, M. P., Junior, C. A. C., & Cortez, M. A. S. 2019.
315 Lactose hydrolysis and organic acids production in yogurt prepared with different onset
316 temperatures of enzymatic action and fermentation. *Ciência Animal Brasileira*, 20, 1–10.
317 <https://doi.org/10.1590/1809-6891V20E-43549>
- 318 8. Batool, Z., Chen, J. H., Liu, B., Chen, F., & Wang, M. 2023. Review on Furan as a Food
319 Processing Contaminant: Identifying Research Progress and Technical Challenges for Future
320 Research. *Journal of Agricultural and Food Chemistry*, 71(13), 5093–5106.
321 <https://doi.org/10.1021/ACS.JAFC.3C01352>
- 322 9. Cheng, H. 2010. Volatile Flavor Compounds in Yogurt: A Review. *Critical Reviews in*
323 *Food Science and Nutrition*, 50(10), 938–950. <https://doi.org/10.1080/10408390903044081>
- 324 10. Eker, T. and T. Cabaroğlu (2020). The Impact factors of yogurt matrix on in vivo aroma
325 release during consumption of yogurt. *GIDA* (2020) 45 (1): 50-60. doi:
326 10.15237/gida.GD19103.
- 327 11. Elbarbary, H. A. 2014. Using of some bifidobacteria species as biopreservative cultures
328 in some dairy products. *Assiut Veterinary Medical Journal*, 60(143), 54–60.
329 <https://doi.org/10.21608/AVMJ.2014.171052>
- 330 12. Fadhlurrohman I, Sumarmono J, Tianling M, Prasetya R, Safitri A, Kafa UA, et al.
331 (2023). Physical and Chemical Properties of Cow's Milk Yogurt Added Whey Protein
332 Concentrate (WPC). *Proceeding ICMA-SURE [Internet]* ;2(1):109–109.
- 333 13. Farooq, K., & Haque, Z. U. 1992. Effect of Sugar Esters on the Textural Properties of
334 Nonfat Low Calorie Yogurt. *Journal of Dairy Science*, 75(10), 2676–2680.
335 [https://doi.org/10.3168/jds.S0022-0302\(92\)78029-1](https://doi.org/10.3168/jds.S0022-0302(92)78029-1)
- 336 14. Guo, J., Zhao, R., Li, J., Wu, D., Yang, Q., Zhang, Y., & Wang, S. 2019. Furan
337 formation from ingredient interactions and furan mitigation by sugar alcohols and antioxidants
338 of bamboo leaves in milk beverage model systems. *Journal of the Science of Food and*
339 *Agriculture*, 99(11), 4993–4999. <https://doi.org/10.1002/JSFA.9739>
- 340 15. He, B. L., Xiong, Y., Hu, T. G., Zong, M. H., & Wu, H. 2023. Bifidobacterium spp. as
341 functional foods: a review of current status, challenges, and strategies. *Crit. Rev. Food Sci.*
342 *Nutrition*. 63, 8048–8065. <https://doi.org/10.1080/10408398.2022.2054934>
- 343 16. Hussain, S. A., Panjagari, N. R., Singh, R. R. B., & Patil, G. R. 2010. Production and
344 function of dealcohol yogurt beverage. *China Dairy Industry*, 38(1), 26–28.

345 <https://doi.org/10.1080/10408398.2011.649148>

346 17. Jegal, S., BCIT School of Health Sciences, Environmental Health, Heacock, H., Trmcic,
347 A., & Shaw, F. 2019. The effect of probiotics as a starter culture for producing yogurt. *BCIT*
348 *Environmental Public Health Journal*. <https://doi.org/10.47339/EPHJ.2019.41>

349 18. Kamara, D. S., Rachman, S. D., Pasisca, R. W., Djajasoepana, S., Suprijana, O., Idar,
350 I., & Ishmayana, S. (2016). Pembuatan dan Aktivitas Antibakteri Yogurt Hasil Fermentasi Tiga
351 Bakteri (*Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*). *Al-*
352 *Kimia*, 4(2), 121–131. <https://doi.org/10.24252/AL-KIMIA.V4I2.1680>.

353 19. Krastanov A, Georgiev M, Slavchev A, Blazheva D, Goranov B, Ibrahim SA. (2023).
354 Design and Volatile Compound Profiling of Starter Cultures for Yogurt Preparation. *Foods*
355 2023, 12(2), 379; <https://doi.org/10.3390/foods12020379>

356 20. Latif, A., A. Shehzad, S. Niazi, A. Zahid, W. Ashraf et al. 2023. Probiotics: mechanism
357 of action, health benefits and their application in food industries. *Front Microbiol.* 2023; 14:
358 1216674. DOI: 10.3389/fmicb.2023.1216674

359 21. Li, H., Gao, J., Chen, W., Qian, C., Wang, Y., Wang, J., & Chen, L. 2022. Lactic acid
360 bacteria isolated from Kazakh traditional fermented milk products affect the fermentation
361 characteristics and sensory qualities of yogurt. *Food Science and Nutrition*, 10(5), 1451–1460.
362 <https://doi.org/10.1002/FSN3.2755>

363 22. Liu, A., Liu, Q., Bu, Y., Hao, H., Liu, T., Gong, P., Zhang, L., Chen, C., Tian, H., & Yi,
364 H. 2022. Aroma classification and characterization of *Lactobacillus delbrueckii* subsp.
365 *bulgaricus* fermented milk. *Food Chemistry: X*, 15, 100385–100385.
366 <https://doi.org/10.1016/J.FOCHX.2022.100385>

367 23. Liu, C., Yang, P., Wang, H., & Song, H. 2022. Identification of odor compounds and
368 odor-active compounds of yogurt using DHS, SPME, SAFE, and SBSE/GC-O-MS. *LWT*, 154,
369 112689. <https://doi.org/10.1016/j.lwt.2021.112689>

370 24. Li, L. Q., Chen, X., Zhu, J., Zhang, S., Chen, S. Q., & Liu, X. 2023. Advances and
371 challenges in interaction between heteroglycans and *Bifidobacterium*: utilization strategies,
372 intestinal health and future perspectives. *Trends Food Sci. Technol.* 134, 112–122.

373 25. Li, L., Zhang, C., Liu, B., Tian, F., Yu, L., Chen, W., & Zhai, Q. 2024. Factor involved
374 in the abundant dominance of *Bifidobacterium logum* within the genus in the human gut. *Food*
375 *Biosci.* 61, 104638. <https://doi.org/10.1016/j.fbio.2024.104638>.

376 26. Nadal, I., Rico, J., Pérez-Martínez, G., Yebra, M. J., & Monedero, V. 2009. Diacetyl
377 and acetoin production from whey permeate using engineered *Lactobacillus casei*. *Journal of*
378 *Industrial Microbiology and Biotechnology*, 36(9), 1233–1237.

379 <https://doi.org/10.1007/S10295-009-0617-9/FULLTEXT.HTML>

380 27. Nair, S., & Peerkhan, N. 2022. Formulation of Millet Milk and Herb Extract Enriched
381 Yogurt and to Assess Its Nutritional Characteristics. *Int J life Sci pharma Res* [Internet]. 2022
382 Dec 12 [cited 2023 Oct 24]; DOI:10.22376/ijlpr.2023.13.1.L151-163 Ni, B., Li, W., Ifrah, K.,
383 Du, B.,

384 28. Ni, B., Li, W., Ifrah, K., Du, B., Xu, Y., Zhang, C., & Li, X. 2022. Dynamic
385 Transcriptome Analysis Reveals Transcription Factors Involved in the Synthesis of Ethyl
386 Acetate in Aroma-Producing Yeast. *Genes*, 13(12), 2341–2341.
387 <https://doi.org/10.3390/GENES13122341>

388 29. Nurhayati, Jayus, J., Rini, A. W. E., Sugiharto, B., & Rahmanto, D. E. 2022. Isolasi dan
389 Identifikasi Khamir Toleran Alkohol dari Molase. *Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati*, 1–
390 10. <https://doi.org/10.24002/BIOTA.V7I1.5426>

391 30. Reineccius, G. 2006 . Flavor Chemistry and Technology. Taylor & Francis. Boca Raton.

392 31. Sakho, M., Crouzet, J., Seck, S., 1985. Volatile components of African mango. *J. Food*
393 *Sci.* 50, 548/550.

394 32. Sharma, M., Wasan, A., & Sharma, R. K. 2021. Recent developments in probiotics: an
395 emphasis on Bifidobacterium. *Food Biosci.* 41, 100993.
396 <https://doi.org/10.1016/j.fbio.2021.100993>

397 33. Shiratsuchi, H., Shimoda, M., Imayoshi, K., Noda, K. & Osajima, Y. 1994. Volatile
398 flavor compounds in spray-dried skim milk powder. *J. Agric. Food Chem.* 42 ; 984-988.

399 34. Siddiqi, M., Taraah, A., Chen, ZH and LaPointe, G. 2024. Phenotypic Differentiation
400 of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. bulgaricus Isolates Found
401 in Yogurt Starter Cultures. *Fermentation.* 10 (12)

402 35. Swelam, S., Rashed, M., & khames, E. 2019. Properties of Non-Fat Yoghurt as
403 Influenced by The Incubation Temperature of Exopolysaccharide Producing Culture. *Journal*
404 *of Food and Dairy Sciences*, 10(12), 447–452. <https://doi.org/10.21608/JFDS.2019.71333>

405 36. Tameko, J. E. M., Chouna, J. R., Nkeng-Efouet-Alango, P., Tapondjou, L. A., &
406 Sewald, N. 2017. Furan derivatives from *Lannea kerstingii*. *Phytochemistry Letters*, 20, 282–
407 284. <https://doi.org/10.1016/j.phytol.2017.04.040>

408 37. Tian, H., Shi, Y., Zhang, Y., Yu, H., Mu, H., & Chen, C. 2019. Screening of aroma-
409 producing lactic acid bacteria and their application in improving the aromatic profile of yogurt.
410 *Journal of Food Biochemistry*, 43(10). <https://doi.org/10.1111/JFBC.12837>

411 38. Tian, H., Yu, B., Yu, H., & Chen, C. 2020. Evaluation of the synergistic olfactory effects
412 of diacetyl, acetaldehyde, and acetoin in a yogurt matrix using odor threshold, aroma intensity,

- 413 and electronic nose analyses. *Journal of Dairy Science*, 103(9), 7957–7967.
414 <https://doi.org/10.3168/jds.2019-17495>
- 415 39. Trentin, M. M., Becker, A. F., Hanauer, D., Bianchi, A., Borges, L., & Schogor, A. L.
416 B. 2022 Development of yogurts with mixture of sheep and bovine milk: effect on chemical
417 physical characteristics, protein profile and antioxidant activity. *Research, Society and*
418 *Development*. <https://doi.org/10.33448/rsd-v11i16.37531>
- 419 40. Vásquez-Trespacios, E. M., & Romero-Palacio, J. 2014. Efficacy of yogurt drink with
420 added plant stanol esters (Benecol® , Colanta) in reducing total and LDL cholesterol in subjects
421 with moderate hypercholesterolemia: a randomized placebo-controlled crossover trial
422 NCT01461798. *Lipids in Health and Disease*, 13(1), 125–125. [https://doi.org/10.1186/1476-](https://doi.org/10.1186/1476-511X-13-125)
423 [511X-13-125](https://doi.org/10.1186/1476-511X-13-125)
- 424 41. Wihansah RRS, Pazra DF, Wahyuningsih, and Handayani KS.(2022). Assesment of the
425 Antidiabetic Activity and Characteristics of Cow’s Milk Yogurt Enhanced with Herbs Extracts.
426 *IOP Conf Ser*. 1020(1):012024–012024.
- 427 42. Wong, K.C. & Tie, D. Y. 1995. Volatile constituent of durians (*Durio ziberthimus*
428 Murr). *J. Flav. and Frag.* 79-83.
- 429 43. Yu, H. S., Lee, N. K., Jeon, H. L., Eom, S. J., Yoo, M. Y., Lim, S. D., & Paik, H. D.
430 2016. Benzoic Acid Production with Respect to Starter Culture and Incubation Temperature
431 during Yogurt Fermentation using Response Surface Methodology. *Korean Journal for Food*
432 *Science of Animal Resources*, 36(3), 427–434. <https://doi.org/10.5851/KOSFA.2016.36.3.427>
- 433 44. Yüksel, A. K., & Bakırcı, İ. 2015. An investigation of the volatile compound profiles of
434 probiotic yogurts produced using different inulin and demineralised whey powder
435 combinations. *Food Science and Biotechnology*, 24(3), 807–816.
436 <https://doi.org/10.1007/S10068-015-0105-0>
- 437 45. Zhang, L., Mi, S., Ruo-bing Liu, Ya-xin Sang, and Xiang-hong Wang. 2020. Evaluation
438 of Volatile Compounds during the Fermentation Process of Yogurts by *Streptococcus*
439 *thermophilus* Based on Odor Activity Value and Heat Map Analysis. *International Journal of*
440 *Analytical Chemistry*. Volume 2020, Article ID 3242854, 10 pages.
441 <https://doi.org/10.1155/2020/3242854>
- 442 46. Zhang, S., & Zhang, L. 2012. Effect of Exopolysaccharide Producing Lactic Acid
443 Bacterial on the Gelation and Texture Properties of Yogurt. *Advanced Materials Research*,
444 430–432, 890–893. <https://doi.org/10.4028/WWW.SCIENTIFIC.NET/AMR.430-432.890>
- 445 47. Zhao, M., Li, H., Zhang, D., Li, J., Wen, R., Ma, H., Zou, T., Hou, Y., & Song, H. 2023.
446 Variation of Aroma Components of Pasteurized Yogurt with Different Process Combination

447 before and after Aging by DHS/GC-O-MS. *Molecules*, 28(4), 1975–1975.

448 <https://doi.org/10.3390/MOLECULES28041975>

449 48. Znamenska, T and O. Vorobiova. 2024. Functions and Individual Mechanisms of
450 Influence of

451 49. Bifidobacteria on The State of Health of Infants (Literary Review). *J. Neonatology,*
452 *Surgery and Perinatal Medicine*. 4(54), pp 191-195