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Screening of Yoghurt Produced by Local Strains of Lactic Acid Bacteria Isolated from Locally Fermented Yoghurt "Kindirmo" For Flavour Compounds

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Abstract

Yoghurt is known as cultured milk which is derived from the action of bacteria on lactose to produce lactic acid, carbon dioxide (CO_2), acetic acid, diacetyl, acetaldehyde, and other flavor compounds. The aim of this research was to screen yoghurt produced by local strains of lactic acid bacteria isolated from locally fermented yoghurt "Kindirmo" for the production of flavor-enhancing compounds. A total of five "Kindirmo" samples were collected from two farmhouses located in Daura local government of Katsina State, Nigeria. The samples were collected and transported in ice containers. Serial dilutions of the "Kindirmo" samples were made and plated using the pour-plate method on de Man, Rogosa, and Sharpe (MRS) agar and incubated anaerobically at 37°C for 72 hours, followed by bacterial identification using Vitek system. Thereafter, yoghurt was produced using the isolated lactic acid bacteria and volatile flavor compounds in the yoghurt were determined using GC-MS analysis. Out of all the five (5) samples analyzed, only two (2) were positive for Lactic acid bacteria. The lactic acid bacteria identified were leuconostoc mesenteroides dextranicum and Lactobacillus acidophilus. While Lactobacillus acidophilus produced 14 volatile flavor compounds, leuconostoc mesenteroides dextranicum produced only 12. Butanoic acid, Hexanoic acid, Acetaldehyde, Propane, Acetone, ethyl ester, Lactic acid, and Diacetone were some of the flavor compounds detected. The research shows the potentials of the isolated LAB to produce flavor compounds, which could be used to enhance the taste of Kindirmo. It is recommended that the isolates should be used for further study on how to produce Kindirmo with single and co-culture of the LAB, or rather on how to produce the flavor enhancers for application in other foods or food products.

Keywords: Lactic acid bacteria, Kindirmo, Flavour, GC-MS, Volatile compounds.

INTRODUCTION

The utilization of lactic acid bacteria (LAB) in the production of cheese, yoghurt, kefir, and buttermilk has significant industrial significance. The species utilized are from the genus *Lactobacillus*, as well as those from the genera streptococcus, lactococcus, Leuconostoc, and *Pediococcus*. These bacteria are distinguished by their fermentative activity, ability to enrich nutrients, improve organoleptic qualities, enhance food safety, and provide positive health effects (Kok and Hutkins 2018). Yoghurt, primarily produced by lactic acid bacteria, is a widely recognized fermented dairy product. Global yoghurt consumption is increasing due to its health benefits (Yuksel and Bakirci, 2015). One of the earliest methods used by humans to transform milk into long-lasting products is the production of yoghurt. Yoghurt's sensory attributes encompass its flavor, color, and texture. Among these characteristics, flavor is a critical factor influencing consumer acceptance and preference for yoghurt (Cheng, 2010). The chemical senses of taste and smell are key determinants of flavor. Taste is the sensation perceived when a substance in the mouth chemically interacts with taste receptors on taste neurons, resulting in perceptions of acidity, sweetness, saltiness, or bitterness (Cheng, 2010). Flavor, being a crucial aspect of food products, significantly influences consumer acceptance and preference.

The sensory attributes of dairy products are greatly influenced by the proportion of flavor components derived from proteins, fats, or carbohydrates in milk. Over ninety (90) distinct volatile chemicals have been identified in yogurt, including glucose, aldehydes, ethanol, ketones, acids, ester compounds, lactones, compounds with sulfur, pyrazines, and furan derivatives.

According to Routray and Mishra (2011), acetoin, 2-butanone, lactic acid, acetaldehyde, and diacetyl are the principal flavoring agents commonly found and documented in yogurt.

Microorganisms in starter cultures produce flavor compounds in yogurt through their activities. The majority of the bacteria in these starter cultures are lactic acid bacteria (LAB), including *Lactobacillus* species, leuconostoc species, bifidobacterium species, Streptococcus thermophilus, and lactococcus lactis. Three significant metabolic transformations of these bacteria perform the following tasks during the fermentation of milk components:

- Glycolysis is the process by which carbohydrates are transformed into lactic acid or other metabolites.
- Casein hydrolysis (proteolysis) results in the formation of peptides along with free amino acids.
- Lipolysis is the process by which milk fat breaks down to free fatty acids (Steele *et al.*, 2013).

MATERIALS AND METHODS

Study Area

This study focused on selected farmhouses/factories within Daura Local Government Area in Katsina State. Daura is situated approximately 35 kilometers from Kazaure in Jigawa State, 120 kilometers from Kano metropolis, 74 kilometers from Katsina metropolis, and near the border with the Republic of Niger. Daura serves as an agricultural hub in the region, known for producing maize, millet, groundnut, and guinea corn. The city is predominantly inhabited by the Hausa and Fulani tribes.

Sample Collection

Five (5) samples of locally fermented yogurt were collected from two (2) different farmhouses/factories within Daura for the isolation and identification of Lactic acid bacteria. The samples were collected in sterile transparent plastic containers with covered lids and transported in ice containers to the Department of Microbiology at Umaru Musa Yar'adua University, Katsina, for analysis.

Isolation of Lactic Acid Bacteria

Lactic acid bacteria were isolated using the methods previously described by Ismail *et al*. (2018). Briefly, de Man Rogosa and Sharpe (MRS) agar was autoclaved for 15 minutes at 121°C following the manufacturer's instructions before inoculation. One milliliter of the "*kindirmo*" sample was mixed with nine milliliters of sterile peptone water (0.1 w/v %) and vigorously shaken for one minute, then serially diluted into five (5) dilutions. Subsequently, 1mL of the appropriately diluted samples was plated on MRS agar using the pour-plate method. The MRS plates were anaerobically incubated for 72 hours at 37°C to observe colony development. Pure cultures of lactic acid bacteria were obtained through repeated sub-culturing on MRS agar. These pure cultures were streaked on agar slants and stored at 4°C for further analysis.

Identification of the Isolated Bacteria Using Vitek system

The vitek-2 Compact machine was used for the automated identification of the bacterial isolates. Controlled suspensions of the pure colonies in saline were inoculated into machine carts containing biochemical broths, with a negative control to evaluate the suspension's development and sustainability. An optical scanner was used to detect if each well was positive or negative by measuring light attenuation. After an incubation period of 5 to 8 hours, the complete reactions were analyzed automatically, and the identification results were printed out (Biomerieux, 2022).

Production of Yoghurt Using Selected Starter Cultures

A McFarland Standard of 0.5 (1.5 × 10^8 CFU/mL) was used as a guide to prepare the lactic acid bacterial suspension of 10^5 CFU/mL as the inoculum size, spectrophotometrically. For the production, sterile glass bottles containing 100mL of raw cow milk samples were pasteurized at 85°C for 30 minutes using a water bath, then cooled to 45°C. The pasteurized cow milk samples were inoculated with 1.0 mL each of separate starter cultures of *L. acidophilus* and *L.* mesenteroides dextranicum at the described inoculum size (10^5 CFU/mL) followed by incubation for five hours at 45°C. Subsequently, the setup was cooled to 5°C, and the yoghurt produced was stored.

GC-MS Analysis for Determination of Flavour Compounds in the Produced Yoghurt

Samples were properly prepared to ensure accurate and reliable results using an extraction and injection method. The injected sample was then separated through gas chromatography, where it was vaporized and passed through a column with a stationary phase to separate analytes based on their molecular weight and charge properties. The final step in GC-MS analysis involved data analysis, where mass spectra obtained from the mass spectrometer were compared based on molecular weight. Quantitative analysis was also conducted by comparing peak areas of the analytes with known standards (Boyd *et al.*, 2018).

Statistical Analysis

In order to ascertain if there is a statistically significant variation in the synthesis of flavour components in yoghurts made with *Lactobacillus acidophilus* and *L. mesenteroides dextranicum*, the Kruskal-Wallis H test was used.

RESULTS

A total of five (5) samples of locally fermented yoghurt *Kindirmo* were collected from two different farmhouses, out of which two (2) were found positive for Lactic acid bacteria. The other three (3) samples were found to harbor bacteria that were different from Lactic Acid bacteria. Table 1 demonstrates the morphology of the bacteria isolated from locally fermented yoghurt (*Kindirmo*) using culture and microscopy. The two (2) Lactic Acid Bacteria found are leuconostoc mesenteroides dextranicum and Lactobacillus acidophilus.

Table 2 indicates the biochemical identification of the bacteria isolated. *Leuconostoc mesenteroides dextranicum* and *Lactobacillus acidophilus* are gram positive rod, oval and spherical shaped bacteria 0.5 to 0.7 µm in diameter and has a length of 0.7 to 1.2 µm. They produce colonies with an average diameter of less than 1.0 mm and lengthy chains of short rods with rounded ends. They are catalase negative, indole negative, urease negative, oxidase negative, non-spore-forming and non-motile.

Table 3 shows the volatile flavour compounds produced by *Lactobacillus acidophilus* which include Propane, Butanoic acid, 1-ethenylhexyl ester, Hexanal, 3-methyl, Butyric acid, neopentyl ester, Propanoic acid, 2-hydroxy-2-methyl, cis-3-Hexenyllactate,2-Pentanone,4hydroxy-4-methyl, Benzoic acid, 4(2,4dimethoxy-6-pentylbenzoyl)oxy,2-methoxy-6-pentyl, methyl ester, Diacetone-D-galacturonic acid, tert-butyldimethylsilyl ester, Acetaldehyde, Tributyrin, Butanoic acid, 2,2-diethyl 3-oxo, ethyl ester, Acetamide, 1-acetoxy-1-phenyl-2-propyl and 2-Pentanone, 4-hydroxy-4-methyl.

Table 4 specified the volatile flavour compounds produced by *Leuconostoc mesenteroides dextranicum* in the produced yoghurt. These include: Hexanoic acid, 1,1-dimethylethyl ester, Hexanoic acid, 1-ethenyl-1,5-dimethyl-4-hexenyl ester, 1-Octen-1-ol, acetate, Sucrose octaacetate, Butanoic acid, 3-hexenyl ester, Propanoic acid, 2-hydroxy-2-methyl-, ethyl ester, acetaldehyde, Diacetylacetone, 2,4,6-Heptanetrione, 1,3-Butadiyne, Butanoic acid, 1,1-dimethyl-2-phenylethyl ester, Butanoic acid, 2-octyl ester and retinoic acid,methylester.

To evaluate whether the identity of the bacterium used in producing the yoghurt influences the amount/variety of flavour compounds in the yoghurt produced, Kruskal-Wallis H test was carried out as described in the preceding chapter, using AnalyStat. Figure 1 presented the result of the statistical comparison, which showed that the yoghurt produced using *Lactobacillus acidophilus* does not produce a statistically significant higher number of flavour compounds (14), compared to yoghurt produced from *L. mesenteroides dextranicum*(12) at 95% confidence interval (p = 0.082, Hcal = 5.0, Critical x^2 = 5.99).

Table 1: Morphology of the Bacteria Isolated from Locally Fermented Yoghurt (*Kindirmo*).

Growth Medium	Colony Morphology	Microscopy	Tentative Identity
MRS-Agar	Occur singly, in pairs, and in short chains	Gram +ve, rod	*Lactobacillus acidophilus*
MRS-Agar	white colonies, short with rounded ends in long chains	Gram +ve, rod shaped	*Leuconostoc mesenteroides dextranicum*

Table 2: Biochemical Characteristics of the Bacteria Isolated

Strain		A	B
Gram reaction		+ve	+ve
Biochemical Tests and Reactions	amy	+ve	-ve
	appa	-ve	-ve
	alaa	+ve	-ve
	drib	-ve	-ve
	novo	+ve	+ve
	draf	-ve	-ve
	opto	-ve	+ve
	piplc	-ve	-ve
	cdex	-ve	-ve
	proa	-ve	-ve
	tyra	-ve	-ve
	llatk	+ve	+ve
	nc6.5	-ve	-ve
	0129r	-ve	-ve
	dxyl	+ve	-ve
	aspa	-ve	-ve
	bgurr	-ve	-ve
	dsor	-ve	-ve
	lac	+ve	-ve
	dman	-ve	-ve
	sal	+ve	-ve
	adh1	-ve	+ve
	bgar	-ve	-ve
	agal	+ve	-ve
	ure	-ve	-ve
	nag	+ve	-ve
	dmne	+ve	+ve
	sac	+ve	-ve
	bgal	+ve	-ve
	aman	+ve	-ve
	pyra	-ve	-ve
	polyb	-ve	+ve
	dmal	+ve	-ve
	mbdg	-ve	-ve
	dtre	-ve	-ve
	aglu	-ve	-ve
	phos	-ve	-ve
	bgur	-ve	-ve
	dgal	+ve	-ve
	bacl	-ve	+ve
	pul	-ve	-ve
	adh2s	-ve	-ve
Organism Isolated		*L. Acidophilus*	*L. Mesenteroides dextranicum*

KEYS:
+ve = positive
-ve = negative
amy= d-amygdalin
appa= ala-phe-proarylamidase
leua= leucinearylamidase
alaa= alanine arylamidase
drib= d-ribose
novo= novobiocin resistance
draf= d-rafficin resistance
opto= optochin resistance
piplc= phosphatidylinostol phospholipase
cdex= cyclodextrine
proa= l-prolinearylamidase
tyra= tyrosine arylamidase
llatk= l-lactate alkalinisation
nc6.5= growth in 6.5% nacl
0129r= 0/129 resistance (comp.vibro.)
dxyl= d-xylose
aspa=l-aspartate arylamidase
bgurr=beta glucuronidase
adh1=arginine dihydrolase 1
bgar=beta galactopyranosidaseresorufine
agal=alpha-galactosidase
ure=urease
nag=n-acetyl-d-glucosamine
dmne=d-mannose
sac=saccharose/sucrose
bgal=beta-galactosidase
aman=alpha-mannosidase
pyra=l-pyrrolydonyl- arylamidase
polyb=polymixin b resistance
dmal=d-maltose
mbdg=methyl-blglucopyranoside
dtre=d-trehalose
aglu=alpha-glucosidase
phos=phosphatase
bgur=beta-glucoronidase
dgal=d-glactose
bacl=bactiracin resistance
pul=pullulane
adh2s=arginine dihydrolase 2
dsor=d-sorbital
lac=lactose
dman=d-mannitol
sal=salicin

Table 3: Volatile Compounds Obtained from Yoghurt Produced with *L. Acidophilus*.

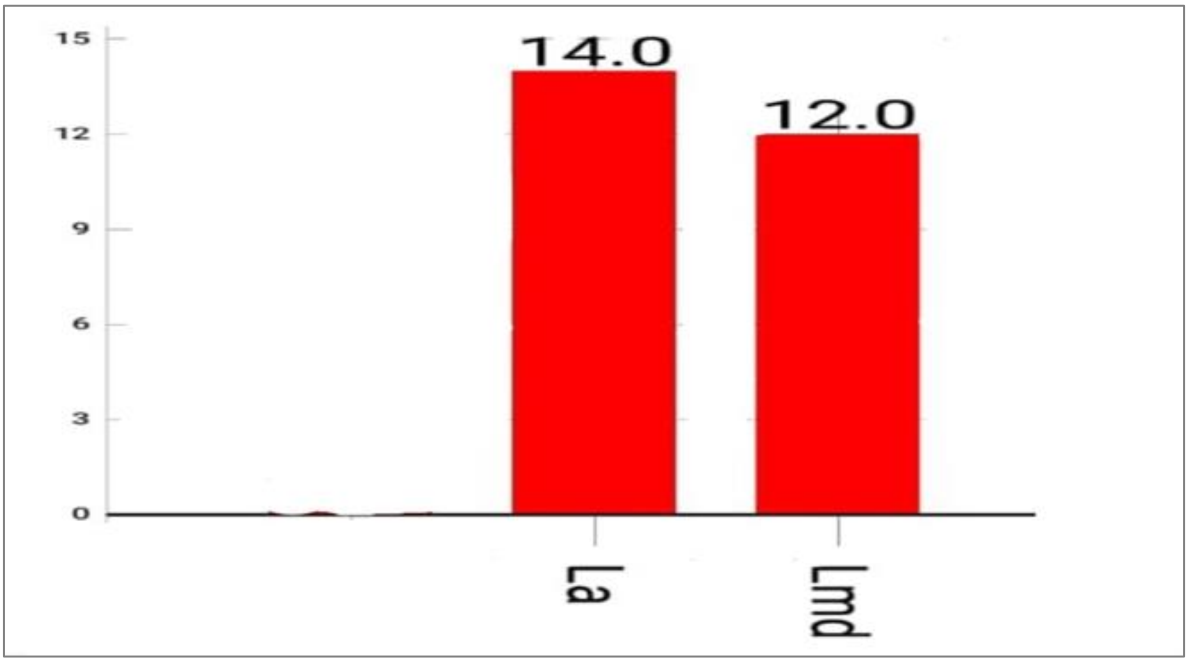
S/N	R.T. (min)	Peak height	Volatile compounds	Group
1.	3.983	1538	Propane	Alkyl
2.	5.490	5547	Butanoic acid, 1-ethenylhexyl ester	Carboxylic acid
3.	6.025	1036	Hexanal, 3-methyl	Aldehyde
4.	7.265	19205	Butyric acid, neopentyl ester	Carboxylic acid
5.	16.700	2032	Propanoic acid, 2-hydroxy-2-methyl	Carboxylic acid
6.	16.769	1343	cis-3-Hexenyllactate	Carboxylic ester
7.	17.229	1581	2-Pentanone, 4-hydroxy-4-methyl	Ketones
8.	19.225	1505	Benzoic acid, 4(2,4-dimethoxy-6-pentylbenzoyl)oxy, 2-methoxy-6-pentyl, methyl ester	Carboxylic acid
9.	20.523	1244	Diacetone-D-galacturonic acid, tert-butyldimethylsilyl ester	Ketones and Aldehyde
10.	21.169	1125	Acetaldehyde	Carbonyl
11.	24.126	2129	Tributyrin	Carboxyl
12.	24.464	1850	Butanoic acid, 2,2-diethyl 3-oxo, ethyl ester	Carboxylic acid
13.	25.144	1387	Acetamide, 1-acetoxy-1-phenyl-2-propyl	Carboxylic acid amide
14.	25.296	1126	2-Pentanone, 4-hydroxy-4-methyl	Ketone

RT = Retention time, min = minutes

Table 4: Volatile Compounds Obtained From Yoghurt Produced with *L. Mesenteroides Dextranicum*.

S/N	R.T. min	Peak height	Volatile compounds	Group
1.	4.733	4904	Hexanoic acid, 1,1-dimethylethyl ester	Carboxylic acid
2.	8.610	60005	Hexanoic acid, 1-ethenyl-1,5-dimethyl-4-hexenyl ester	Carboxylic acid
3.	11.461	1945	1-Octen-1-ol, acetate	Alkynes
4.	12.067	2569	Sucrose Octaacetate	Hydroxyl and Acetal group
5.	13.382	6060	Butanoic acid, 3-hexenyl ester	Carboxylic acid
6.	13.545	2668	Propanoic acid, 2-hydroxy-2-methyl-, ethyl ester	Carboxylic acid
7.	16.723	3059	Acetaldehyde	Aldehyde
8.	17.642	1651	Diacetylacetone, 2,4,6-Heptanetrione	Ketone
9.	20.506	1307	1,3-Butadiyne	Alkene
10.	20,680	3191	Butanoic acid, 1,1-dimethyl-2-phenylethyl ester	Carboxylic acid
11.	21.961	1266	Butanoic acid, 2-octyl ester	Carboxylic acid
12.	22.747	2380	Retinoic acid, methyl ester	Carboxylic acid

RT = Retention time, min = minute



Lactic Acid Bacterial Strains used in Yoghurt Production

Figure 1: Statistical Comparison in Production of Flavour Compound in Yoghurts Produced Using the Various Lactic acid bacteria.

Keys:

LA= *Lactobacillus acidophilus*

LMD= *Leuconostoc mesenteroides dextranicum*

DISCUSSIONS

In this study, *Kindirmo*, a locally fermented yogurt, contains specific Lactic acid bacteria responsible for fermentation and the production of volatile compounds. The identified Lactic acid bacterial species from *Kindirmo* are *Lactobacillus acidophilus* and *Leuconostoc mesenteroides dextranicum*. Traditionally, Lactobacillus delbrueckii bulgaricus and Streptococcus thermophilus are commonly used as starter cultures for yogurt production (Zong *et al.*, 2022). This aligns with the findings of Sudi *et al.*, (2008), who also identified *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in locally fermented yoghurt like *Kindirmo*.

According to Kok and Hutkins (2018), yogurt made with *Lactobacillus bulgaricus* and *Streptophilusthermophillus* is believed to promote the beneficial microbiota of the gut, even though these bacteria are not typically found there, contributing to overall intestinal health. On the other hand, consuming probiotic-rich yogurt is said to offer a variety of health benefits by introducing beneficial microbes, such as *L. acidophilus* and *Bifidobacteria*. It was noted that *Lactobacillus* acidophilus, the predominant lactic acid bacterium in locally fermented yogurt kindirmo, was responsible for producing fourteen volatile compounds, primarily carboxylic acids and ketones, while *Leuconostoc mesenteroides dextranicum* produced twelve volatile compounds.

The most common volatile compounds produce by almost all Lactic acid bacteria in our study are; Butanoic acid, Hexanoic acid, Acetaldehyde, Propane, Acetone, Ethylester, Lactic acid and Diacetone, with Hexanoic acid, 1, 1-dimethylethyl ester having the highest peak compared to the other volatile compounds present in yoghurt produced with *L. acidophilus* as starter culture, Butyric acid, neopentyl ester was the volatile compound having the highest peak height, and Hexanal; 3-methyl has the least peak height. It was also discovered that Hexanoic acid, 1-ethenyl -1,5-dimethyl -4-hexenyl ester had the highest peak height in yoghurt produced using *L. Mesenteroides dextranicum*as starter culture, while Butanoic acid, 2-octyl ester has the least peak height among the volatile compounds produced. Yoghurt's acceptance and preference are largely determined by its flavour. As mentioned earlier, local yoghurts have unknown flavours which can have prospects. Compounds similar to those reported in this study were reported from the work of Cheng (2010), namely: were Acetaldehyde, Acetone, Propane, Diacetyl, 2-Hexanone, Acetic acid, Butanoic acid, Propanoic acid, and Ethylacetate. By and large, these are the most common volatile compounds produced by Lactic acid bacteria in yoghurt. Another study revealed that the greatest fat content yoghurt was best praised for its texture and flavour. There were sixteen (16) volatile flavoring substances detected, according to Kaminarides *et al*. (2007). This research showed that statistically, the specific type of Lactic acid bacteria has no significant impact on flavour compounds that are produced; therefore, one may employ any of the lactic acid bacteria that have been identified to produce yoghurt as they have the ability to produce enough flavour compounds. It was observed that local starter cultures produce large number of volatile compounds during the yoghurtproduction compared to those reported in yoghurts produced using commercial starters and these compounds are advantageous in increasing the shelf life of yoghurt during storage (Zhang *et al*., 2020).

CONCLUSIONS

This study identified some lactic acid bacteria that produce volatile flavor compounds in yogurt, including *Lactobacillus* acidophilus, which produced a large number of volatile flavor compounds such as Butanoic acid, Hexanoic acid, Acetaldehyde, Propane, Acetone, Ethylester, Lactic acid, and Diacetone.

REFERENCES

Biomerieux (2022).*Vitek2systemBrochure*. *www.biomerieuxusa.com* brochure_v2.pdf.

Boyd, C. C., Cheacharoen, R., Leijtens, T., and McGehee, M. D. (2018).Understanding degradation mechanisms and improving stability of perovskite photovoltaic.*Chemical reviews*, 119(5), 3418-3451. **[Crossref]**

Cheng, H., (2010). Volatile Flavour Compounds in Yoghurt: A Review. *Critical Reviews in Food Science and Nutrition*, 50, 938-950. **[Crossref]**

Ismail, Y. S., Yulvizar, C., and Mazhitov, B., (2018). Characterization of lactic acid bacteria from local cow s milk kefir. *In IOP Conference Series: Earth and Environmental Science (Vol. 130, p. 012019). IOP Publishing*. **[Crossref]**

Kaminarides S, Stamou P, and Massouras T., (2007). Comparison of the characteristics of set-type yoghurt made from ovine milk of different fat content. *Int. J Food SciTechnol*42(9):1019–28. **[Crossref]**

Kok, C. R., Hutkins, R., (2018). Yoghurt and Other Fermented Foods as Sources of Health- promoting Bacteria.*Nutr. Rev. 2018, 76(Suppl 1),* 4–15. DOI: 10.1093/nutrit/nuy056. **[Crossref]**

Routray, W., Mishra, H.N., (2011). Scientific and Technical Aspects of Yoghurt Aroma and Taste: A Review. *Comprehensive Reviews in Food Science and Food Safety*, 10, 208–220. **[Crossref]**

Settachaimongkon, S., Nout, M. J. R., Fernandes, E. C. A., Hettinga, K. A., Vervoort, J. M., van Hooijdonk, T. C. M., *et al*. (2014). Influence of different proteolytic strains of *streptococcus thermophilus* in co-culture with *Lactobacillus delbrueckiisubspbulgaricus* on the metabolite profile of set-yoghurt. *International Journal of Food Microbiology*, 177, 29–36. **[Crossref]**

Steele, J., Broadbent, J., Kok, J., (2013). Perspectives on the Contribution of Lactic Acid Bacteria to Cheese Flavour Development.*Current Opinion in Biotechnology*, 24, 135-141. **[Crossref]**

Sudi, I. Y., De, N., and Ali-Dunkrah, U., (2008).Mutagenesis and selection of *Lactobacillus bulgaricus*and *Streptococcus thermophiles* for potential use as starter culture.*Journal of American Sciences*, 4(3), 80-87.

Yuksel, A. K., and Bakirci, I., (2015). An investigation of the volatile compound profiles of probiotic yoghurts produced using different inulin and demineralised whey powder combinations. *Food Science and Biotechnology, 24(3), 807-816*. **[Crossref]**

Zhang, S. S., Xu, Z. S., Qin, L. H., and Kong, J., (2020).Low-sugar yoghurt making by the co- cultivation of Lactobacillus plantarum WCFS1 with yoghurt starter cultures.*Journal of dairy science, 103(4), 3045-3054*. **[Crossref]**

Zong, L., Lu, M., Wang, W., Wa, Y., Qu, H., Chen, D., and Gu, R., (2022). The Quality and Flavour Changes of Different Soymilk and Milk Mixtures Fermented Products during Storage. Fermentation-Basel, 8(12). **[Crossref]**