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Production of flavour compounds by yogurt starter cultures

D Beshkova, E Simova, G Frengova and Z Simov

Laboratory of Applied Microbiology, Institute of Microbiology, Bulgarian Academy of Sciences, 4002 Plovdiv, 26 Maritza Blvd, Bulgaria

The present work studied the production of carbonyl compounds and saturated volatile free fatty acids by pure cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, and by starter cultures for Bulgarian yogurt during cultivation and cooling. The mixed cultures formed volatile aromatic compounds more actively than the pure cultures. A guiding factor in the preparation of the starter cultures was the biochemical activity of *Lactobacillus bulgaricus* in synthesizing the major carbonyl compounds, acetaldehyde, diacetyl and the volatile fatty acids C_2–C_{10}. The activity of the yogurt cultures in synthesizing carbonyl compounds was at its highest during milk coagulation and cooling, up to 7 h. However, maximum concentration was reached by 22–31 h. In the cooled 22–h starter cultures, acetaldehyde predominated (1415.0–1734.2 μg per 100 g) followed by diacetyl (165.0–202.0 μg per 100 g), acetoin (170.0–221.0 μg per 100 g), acetone (66.0–75.5 μg per 100 g), ethanol (58.0 μg per 100 g), and butanone-2 (3.6–3.8 μg per 100 g). The thermophilic streptococcus and lactobacillus cultures, and the starter cultures contained predominantly acetic, butyric and caproic acids.

Keywords: *Streptococcus thermophilus*; *Lactobacillus bulgaricus*; carbonyl compounds; free fatty acids; yogurt

Introduction

The compounds which impart the distinctive flavour to yogurt are lactic acid and a variety of volatile organic aroma compounds, produced by *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Although a long list of volatile organic compounds has been identified in yogurt [31], in most investigations only a few of them proved to have a decisive influence on the resulting aroma, because of their comparatively high concentration. In screenings of starter cultures for yogurt and yogurt-related products, precisely these compounds are included [18,33]. In their study, Imhof *et al* [15] considered only five of the identified 33 volatile compounds as influencing the flavour of yogurt with a thermophilic starter culture. A greater number of the volatile organic compounds identified in yogurt are not produced by the starter bacteria but originate from the milk (aldehydes, ketones and alcohols) [15,30].

Acetaldehyde is recognized as a major flavour component in yogurt [3,9,20] and the evaluation of its flavour, according to some authors, is based mainly on acetaldehyde production by the starter culture [4,5]. Some authors proved the availability of threonine aldolase, a catalyst of acetaldehyde formation from threonine, only in *Lactobacillus bulgaricus* [27]; other researchers found it in both thermophilic lactobacteria [21,23,32]. The low contents of acetaldehyde in goat's yogurt, which is richer in glycine than cow's yogurt, could be accounted for by the inhibition of threonine aldolase by glycine [29].

There are differing views on the role of diacetyl in the formation of yogurt flavour. Some authors regard it as a dominating flavour component only when acetaldehyde contents are low [13,28] while others attribute to it the leading part in flavour formation [15,18]. According to Rasic and Kurmann [25], *Streptococcus thermophilus* is solely responsible for the availability of diacetyl in yogurt whereas other authors reported that diacetyl was also largely produced by *Lactobacillus bulgaricus* [10].

Lactic acid and the volatile fatty acids (saturated low-molecular) are components with a specific role in the aroma and flavour properties of yogurt [12,26]. The acid-forming activity of the starter culture is dependent on the strains in the association, the lactobacillus in particular [4,5]. Despite the significant progress in chromatographic methods (headspace analyses), which have improved the response in detecting volatile compounds in fermented milks [7,16], scientific research in this direction has given only few and rather controversial results.

In this study we investigated the production of carbonyl compounds and saturated volatile fatty acids by pure cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, and by two starter cultures during the cultivation and cooling stages of Bulgarian yogurt manufacture. Both strains in the starter culture are biologically compatible and capable of forming the typical yogurt aroma and flavour when grown in association. In our selection of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* strains we accentuated those strains which had been isolated from original yogurt products, where the thermophilic streptococcus and lactobacillus had been coexisting for a long time and had established their own biological equilibrium. The two starter cultures thus formed, and the yogurt manufactured from them were found to have satisfactory aroma and flavour characteristics. Both starter cultures were used in commercial production of Bulgarian yogurt for 2 years.

Correspondence: ED Simova, Laboratory of Applied Microbiology, Institute of Microbiology, Bulgarian Academy of Sciences, 4002 Plovdiv, 26 Maritza Blvd, Bulgaria
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Materials and methods

Microorganisms

Thirty-two *L. bulgaricus* strains and fourteen *S. thermophilus* strains were isolated from home-made cow's and sheep's milk yogurts, manufactured in certain mountain regions in Bulgaria. The yogurts possessed viable lactic acid microflora and excellent specific organoleptics typical of Bulgarian yogurt. These fermented milks had been used in the households as starter cultures for 8–10 months. In the continual use of milks for starter cultures the lactic acid microflora was subjected to natural selection. In this way the species formed stable symbiotic relationships and were able to manifest their typical properties repeatedly in natural combinations. The lactobacilli and thermophilic streptococci strains were affiliated to the species *Lactobacillus delbrueckii* subsp *bulgaricus* and *Streptococcus salivarius* subsp *thermophilus* according to Bergey's determinant, 1986.

These strains were used to form 80 associations, which were all tested as starter cultures for yogurt. Having studied the morphology of the cultures, the ratio of the two strains, the starter cultures' activity, and the organoleptic properties of the manufactured yogurt, we selected two starter cultures: *S. thermophilus* 13a + *L. bulgaricus* 2–11 and *S. thermophilus* 15a + *L. bulgaricus* 1–9. These starters composed by a mixing of pure cultures were obtained in the following way: whole cow's milk, sterilized and cooled to 45°C was inoculated with 1% (v/v) of *S. thermophilus* + 1% (v/v) of *L. bulgaricus*, then incubated at 45°C. The morphology and cell ratio of both microorganisms in the starter cultures were determined microscopically, and the number of viable cells and coagulation time were also established. The starter cultures with the desired strain ratio (3 : 1 = *S* : *L*) were subjected to daily transfer for 3 months, and weekly transfers for 1 month.

The organoleptic properties of the starter cultures and manufactured yogurt were determined in the following way: whole cow's milk, homogenized, pasteurized at 95°C for 30 min, and cooled to 45°C, was inoculated with 2% (v/v) of 2-h mixed cultures *S. thermophilus* 13a + *L. bulgaricus* 2–11 and *S. thermophilus* 15a + *L. bulgaricus* 1–9. The milks were thermostated at 45°C until coagulation (pH = 4.5–4.6, lactic acid 6.95 g L^{-1}). The coagulated samples were cooled and stored at 6°C. The 12-, 24- and 48-h cooled samples were tested for organoleptic properties, texture, coagulum structure, flavour and aroma. In these mixed cultures we established the ability of the lactobacilli and thermophilic streptococci to co-exist in a steady continuance during the 1-year investigation, and to retain the desirable ratio (3 : 1 = *S* : *L*) during repeated transfers. The organoleptic, physiological and biochemical properties of both starter cultures were consistent with prime starter cultures for Bulgarian yogurt: milk coagulation time: 1.5–2.0 h; thick coagulum with bright fracture; coagulum texture and structure after breaking: uniform, grain-free, homogeneous, cream-like; flavour and aroma: strictly specific, distinctly lactic-acid; acidity: lactic acid 7.44 g L^{-1} (pH = 4.3–4.4); microscope picture: abundance of *S. thermophilus* cells as diplococci, and *L. bulgaricus* as short rods, single and in pairs, or short chains with prevailing streptoccoci; total activity: over 100×10^8 cells ml^{-1}. Both starter cultures were used for commercial manufacture of yogurt for a period of 2 years.

The pure cultures *S. thermophilus* 13a, *S. thermophilus* 15a, *L. bulgaricus* 2–11 and *L. bulgaricus* 1–9, and the starter cultures *S. thermophilus* 13a + *L. bulgaricus* 2–11 and *S. thermophilus* 15a + *L. bulgaricus* 1–9 were maintained in sterilized skim cow's milk in test-tubes by weekly transfers, and stored at 4°C.

Enumeration of microorganisms

Viable cells (in colony forming units: CFU ml^{-1}) were determined from the colony counts on specific lactic agar: Streptococcus selective agar for *S. thermophilus* and LB-agar for *L. bulgaricus*. Serial dilutions of each sample were plated in triplicate and plates were incubated at 37°C until growth of the colonies. The results were the mean of six counts at three different dilutions.

The viable streptococci counts were estimated on plates with a medium of Streptococcus selective agar (Merck, Darmstadt, Germany) with the following composition (g L^{-1}): peptone from casein 14.4; peptone from soymeal 5.0; sodium chloride 4.0; d(+)glucose 5.0; sodium citrate 1.0; l-cystine 0.2; sodium sulfite 0.2; sodium azide 0.2; crystal violet 0.002 and agar-agar 13.0, pH = 7.4.

The viable lactobacilli counts were estimated on plates with a medium of LB-agar (Fluka, Buchs, Switzerland) with the following composition (g L^{-1}): tryptone 10.0; yeast extract 5.0; sodium chloride 5.0 and agar-agar 10.0, pH = 7.2.

Morphological control on the microbial cells was performed with a microscope Laboval 4 (Carl Zeiss Jena, Germany) with 1600× magnification. For an estimation of *S. thermophilus–L. bulgaricus* ratio in the yogurt culture, direct microcopic examination was used, previously staining slide preparations with methylene blue according to Breed's method [6].

Cultivation conditions

The inoculum from *S. thermophilus* 13a, *S. thermophilus* 15a, *L. bulgaricus* 2–11 and *L. bulgaricus* 1–9, and the association *S. thermophilus* 13a + *L. bulgaricus* 2–11 and *S. thermophilus* 15a + *L. bulgaricus* 1–9 were grown in 100-ml Erlenmeyer flasks, containing 60 ml whole cow's milk (dry weight 12.3%, fat content 3.6%, lactose 43.0 g L^{-1}, pH = 6.2–6.3). The milk was sterilized at 115°C for 15 min, cooled to 45–46°C and inoculated with 2% (v/v, $6.0–8.0 \times 10^9$ cells ml^{-1}) of the respective pure and mixed cultures. The inoculated milks were thermostatically incubated at 45°C until milk coagulation and pH = 4.3–4.4, for 4.0 h for *S. thermophilus* 13a and *S. thermophilus* 15a, 4.5 h for *L. bulgaricus* 2–11 and *L. bulgaricus* 1–9, and 2.0 h for the associations.

S. thermophilus 13a, *S. thermophilus* 15a, *L. bulgaricus* 2–11, *L. bulgaricus* 1–9 and the mixed cultures were batch cultivated in a 2.0-L fermenter MBR AG (Zurich, Switzerland) with culture volume 1.3 L, 2% (v/v) inoculum, initial oxygen concentration of milk 20% (carried to that concentration with argon), no oxygen inflow during the fermentations, at 45°C, stirring 250 rpm, duration: 4.0 h for the streptococci; 4.5 h for the lactobacilli and 2.0 h for the

mixed cultures. The fermenter was provided with IMCS-2000 operating control, and controls for stirrer speed, temperature, O_2 and CO_2 concentrations, and pH.

Analytical methods

Chromatographic analyses: The carbonyl compounds were qualitatively and quantitatively determined by dynamic headspace analysis using the procedures of Bassette *et al* [1]: 2.0 g of anhydrous sodium sulfate was transferred into a 5-ml serum vial (15 mm diam. × 52 mm, with self-sealing rubber cap) followed by 2.0 g of the homogeneous yogurt sample. After the vial was sealed with a serum cap, the solution was mixed on a mechanical shaker for 5 min. The vial was then placed for 3 min in a 60°C water bath. A 1-ml sample of the headspace gas was obtained by inserting the needle of the gas-tight syringe through the rubber serum cap and drawing the vapors into the syringe (Hamilton No. 1001). The gas sample was analysed on a gas-chromatograph, Fractovap, model D, Carlo Erba (Milan, Italy), equipped with a stainless-steel column (1800 mm long, 4 mm in diameter), packed with 10% Carbowax-400 on Chromosorb W 60–80 mesh, silanized, and flame-ionization detector. Operating conditions: temperature (°C)—detector 150, injector 150, column 60; carrier gas—nitrogen 40 ml min^{-1}; chart speed, 10 mm min^{-1}.

The free volatile fatty acids C_2–C_{10} were isolated from the fermented milks by steam distillation according to Rachev's method [24], and identified on a gas-chromatograph, Hewlett-Packard (Vienna, Austria) series 7400, with a flame-ionization detector, with the following parameters: column, (2000 × 4 mm) spiral, Pyrex glass; stationary phase, 5% Tween 20; stationary carrier, Varaport 30, 70–80 mesh, silanized; carrier gas, nitrogen, 70 ml min^{-1}; hydrogen, 35 ml min^{-1}; air, 300 ml min^{-1}; initial temperature, 100°C 5 min isotherm; program velocity, 3°C; final temperature, 160°C, 25 min isotherm; detector temperature, 190°C; injector temperature, 210°C.

The free amino acids were isolated from a filtrate after precipitating the proteins with trichloracetic acid according to a method described by Mansour [22], and identified on a H-1200E aminoanalyser (Prague, Czech Republic).

Lactose and lactic acid were determined by enzymatic methods as described by Boehringer Mannheim [2].

Results

In the lactobacillus pure cultures as well as in the starter cultures the prevailing constituent was acetaldehyde (Tables 1 and 2). Both *L. bulgaricus* strains showed good acetaldehyde production ability as early as the first hours of their single and associated growth with the streptococci. During cultivation, cooling and storage, acetaldehyde concentration in the starter cultures was considerably higher than that in the pure *L. bulgaricus* cultures. In the pure cultures significant levels of acetaldehyde were detected after 4 h of growth, but in the mixed cultures they were found after 2.5 h of growth, ie immediately after milk coagulation. Acetaldehyde production continued after milk coagulation during refrigeration and storage of the starter cultures. Maximum concentrations were recorded after 22 h in the mixed cultures with *L. bulgaricus* 2–11, and after 31 h in the mixed cultures with *L. bulgaricus* 1–9. After reaching maximum levels in both starter cultures, acetaldehyde concentration was changing slightly until 46 h, and proceeded to decrease more perceptibly until 168 h. In spite of the higher producing ability of *L. bulgaricus* 1–9, *L. bulgaricus* 2–11 was ahead of *L. bulgaricus* 1–9 in the mixed culture. In *S. thermophilus* 13a + *L. bulgaricus* 2–11 at 1.5 h, acetaldehyde concentration was significantly higher: 410.0 μg per 100 g compared to 285.0 μg per 100 g in the culture with *L. bulgaricus* 1–9, and the maximum occurred 9 h earlier. While *L. bulgaricus* 2–11 cell concentration was growing and pH was correspondingly falling in the mixed culture, we observed intensive production of acetaldehyde by 7 h (Figure 1, Table 1). After full coagulation of milk (2.5 h; lactic acid concentration 7.6 g L^{-1}; pH = 4.36; streptococcus : lactobacillus = 3 : 1), acetaldehyde concentration was sufficient (640.0 μg per 100 g) to evaluate the distinctive aroma of yogurt. The cooled 22-h starter culture (lactic acid 7.98; pH = 4.30; acetaldehyde 1734.2 μg per 100 g) possessed very well pronounced lactic acid flavour and specific aroma.

The thermophilic streptococcus strains manifested relatively much lower activity of acetaldehyde production (Tables 1 and 2). In both *S. thermophilus* pure cultures, acetaldehyde was detected at 4 h of growth with maximum concentration at 22 h.

During acid formation and refrigeration we observed active production of diacetyl by pure *L. bulgaricus* and mixed cultures unlike the streptococcus cultures (Tables 1 and 2). This specificity of two *L. bulgaricus* strains is not typical of the lactobacilli of this species. The residual pyruvate, being toxic for the cells, had probably been utilized, as a result of which neutral compounds like diacetyl and acetoin were formed. So far, detailed studies of diacetyl production by *L. bulgaricus* have not been described in the literature. Diacetyl synthesis by the mixed cultures followed the pattern of diacetyl synthesis by the pure *L. bulgaricus* cultures. Active diacetyl production was observed during the first 4 h in both mixed cultures, ie in the log phase of growth and at the outset of the cooling stage (Tables 1 and 2). The synthesis continued throughout cooling with maximum concentration 220.0 μg per 100 g at 31 h in the starter culture with *L. bulgaricus* 1–9, and 165.0 μg per 100 g at 22 h in the starter culture with *L. bulgaricus* 2–11. Consequently, diacetyl is a determinative factor in the flavour formation of the starter cultures and via them determines the flavour of Bulgarian yogurt. These results were confirmed by evaluating the organoleptic properties of starter cultures and yogurts. Diacetyl contributed to the delicate, full-bodied flavour of yogurt with fresh milk aroma.

In the *L. bulgaricus* 2–11 and *L. bulgaricus* 1–9 pure cultures we found acetone (Tables 1 and 2), which was also available in the *S. thermophilus* 13a pure culture, but not detected in the *S. thermophilus* 15a culture (Tables 1 and 2). *S. thermophilus* pure cultures were most active in synthesizing acetoin, which was not produced by the lactobacilli (Tables 1 and 2). Both cultures produced acetoin during the periods of active fermentation and refrigeration up to 22 h.

Table 1 Production of carbonyl compounds (μg per 100 g) by yogurt cultures during lactic acid fermentation

Fermentation and cooling time (h)	Pure cultures											Starter culture					
	S. thermophilus 13a						*L. bulgaricus* 2–11					*S. thermophilus* 13a + *L. bulgaricus* 2–11					
	Acetaldehyde	Diacetyl	Acetone	Acetoin	Ethanol	Butanone-2	Acetaldehyde	Diacetyl	Acetone	Ethanol	Butanone-2	Acetaldehyde	Diacetyl	Acetone	Acetoin	Ethanol	Butanone-2
1.5	80.0	–	–	63.0	40.0	–	199.1	50.0	10.0	35.0	3.5	410.0	68.0	9.0	81.1	40.0	3.0
2.5	90.0	–	–	74.5	40.0	–	461.2	85.0	28.0	35.0	3.5	640.0	95.0	11.0	82.5	40.0	3.2
4.0	151.1	21.0	31.0	78.0	80.0	2.5	682.0	100.0	40.0	50.0	3.6	801.0	138.0	32.5	90.0	45.0	3.4
7.0	151.8	31.0	35.0	125.0	90.0	3.0	803.3	118.0	56.0	53.0	3.6	1516.5	150.0	69.0	150.0	55.0	3.5
22.0	174.0	33.0	40.0	158.0	95.0	2.5	1101.0	125.0	59.0	53.0	3.6	1734.2	165.0	66.0	170.0	58.0	3.6
31.0	171.0	30.0	40.0	150.0	90.0	–	988.5	125.0	56.0	45.0	3.5	1630.0	162.0	60.0	150.0	55.0	3.5
46.0	169.0	30.0	40.0	100.0	85.0	–	980.0	118.0	40.0	45.0	3.5	1627.5	148.0	46.4	110.0	50.0	3.5
168.0	94.0	–	–	85.0	50.0	–	767.0	80.0	32.0	40.0	3.0	1286.5	95.0	45.0	90.0	40.0	2.5

Carbonyl compounds in sterilized base-milk (μg per 100 g): acetaldehyde, 25.0; diacetyl, 1.6; acetone, 6.0; ethanol, 30.0; butanone-2, 3.6.

Table 2 Production of carbonyl compounds (μg per 100 g) by yogurt cultures during lactic acid fermentation

Fermentation and cooling time (h)	Pure cultures									Starter culture					
	S. thermophilus 15a				*L. bulgaricus* 1–9					*S. thermophilus* 15a + *L. bulgaricus* 1–9					
	Acetaldehyde	Diacetyl	Acetoin	Ethanol	Acetaldehyde	Diacetyl	Acetone	Ethanol	Butanone-2	Acetaldehyde	Diacetyl	Acetone	Acetoin	Ethanol	Butanone-2
1.5	85.0	–	45.0	45.0	97.0	40.0	6.0	40.0	3.5	285.0	45.0	8.5	68.0	35.0	3.5
2.5	95.0	–	58.0	45.0	344.4	78.0	12.0	45.0	3.5	490.0	105.0	18.5	99.5	35.0	3.7
4.0	99.6	–	88.0	70.0	465.0	121.0	37.0	50.0	4.0	630.0	165.0	45.0	151.4	40.0	3.9
7.0	110.0	20.0	175.0	90.0	995.0	155.0	46.0	55.0	3.8	992.0	180.0	73.3	233.4	55.0	3.9
22.0	121.0	28.0	193.0	100.0	1207.0	176.0	50.2	55.0	3.8	1415.0	202.0	75.5	221.0	58.0	3.8
31.0	110.0	30.0	190.0	90.0	1223.0	185.0	48.0	55.0	3.8	1506.0	220.0	75.0	215.0	65.0	3.6
46.0	95.4	30.0	175.0	83.0	1081.0	180.0	44.0	50.0	3.8	1465.0	218.0	63.3	200.0	55.0	3.5
168.0	59.9	–	110.0	45.0	689.0	98.0	37.0	45.0	3.3	1185.0	105.0	49.5	95.0	35.0	3.0

Carbonyl compounds in sterilized base-milk (μg per 100 g): acetaldehyde, 25.0; diacetyl, 1.6; acetone, 6.0; ethanol, 30.0; butanone-2, 3.6.

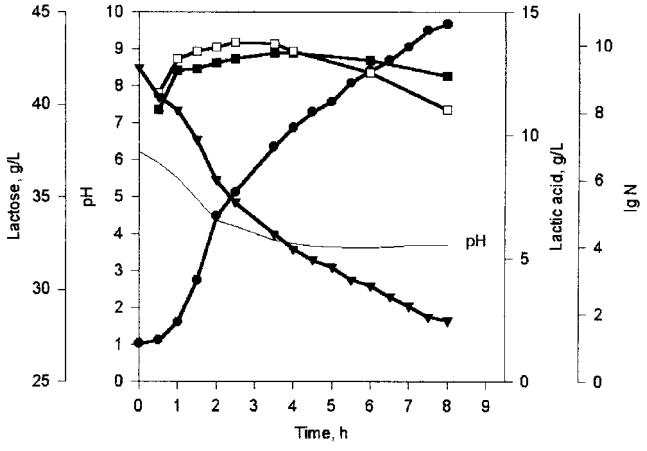

Figure 1 Growth of starter culture *Streptococcus thermophilus* 13a + *Lactobacillus bulgaricus* 2–11 at batch cultivation in bioreactor. (—•—) Lactic acid; (—□—) *Streptococcus* concentration; (—■—) *Lactobacillus* concentration; (—▼—) lactose.

S. thermophilus 13a and *S. thermophilus* 15a produced approximately twice as much ethanol as the *L. bulgaricus* cultures. In the starter cultures the amount of ethanol approaches the amount synthesized by the *L. bulgaricus* pure cultures. In the *S. thermophilus* 15a culture butanone-2 was not detected. The lactobacilli and *S. thermophilus* 13a produced butanone-2 whose concentrations in both starter cultures approximated to those in the *L. bulgaricus* pure cultures.

In spite of certain slight lipolysis in the fermented milks, we determined elevated concentrations of free volatile fatty acids (C_2–C_{10}) in the starter cultures compared with the base sterilized milk. In the cooled 22-h *S. thermophilus* and *L. bulgaricus* pure cultures, and in the starter cultures we found acetic, propionic, butyric, isovaleric, valeric, caproic, caprylic and capric acids (Table 3). The highest concentration was that of the acetic acid followed by the butyric and caproic acids.

Discussion

The mixed cultures stimulated the synthesis of metabolites, which are particularly important for the flavour characteristics of the starter culture. It is noteworthy that, compared to the pure cultures, the starter cultures were significantly enriched with the two flavour-forming compounds, a consequence of the symbiosis between the thermophilic streptococcus and the lactobacillus, which was also observed by other authors [3,14]. The prevalence of the two carbonyl compounds during mixed cultivation originates from the high production activity of both lactobacillus strains in relation to the above basic flavour compounds. The leading role of *L. bulgaricus* observed in the synthesis of acetaldehyde and diacetyl, does not confirm or come anywhere near the reports by authors who recognise *S. thermophilus* as the only producer of diacetyl in yogurt [25] and who detect diacetyl in yogurt only at low aldehyde concentration [13,28]. In other authors' works diacetyl is only in minor concentrations in the thermophilic starter cultures for Bulgarian yogurt [19] and for classical yogurt [15], but is considerably lower than the concentrations defined by us. According to our results the ability of thermophilic streptococci to produce acetaldehyde and diacetyl, though in smaller amounts, does not affect their general synthesis pattern in mixed cultures. When cell concentration increased and pH decreased in the mixed culture during milk coagulation and over the first hours of cooling (up to 7 h), the activity of acetaldehyde and diacetyl intensified—60–90% of the maximum amount of acetaldehyde and diacetyl were produced. The established acetaldehyde and diacetyl concentrations and their ratio (7–10 : 1) were of great importance for the formation of the desirable organoleptic properties in the starter cultures and yogurt, which were recorded throughout the whole production process.

The more prominent presence of acetone in the mixed cultures is mainly due to the lactobacilli. Acetone influences the aroma and flavour qualities of yogurt [30], however, the variety of flavours in different yogurts is recognised basically by the difference in the acetaldehyde : diacetyl ratio [12]. In the mixed cultures acetoin synthesis was about the same as that in the *S. thermophilus* monocultures.

The formation of volatile fatty acids (C_2–C_{10}) was more active in the mixed cultures than in the pure ones owing to the stimulating effect of protocooperation between the two thermophilic species on metabolic activities, which are responsible for the formation of free fatty acids. According to some authors, the hydrolytic activity of *S. thermophilus* towards milk fat is low [8,30]; others registered intracellular esterases in *L. bulgaricus*, with insignificant variations

Table 3 Free volatile fatty acids in 22-h pure cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, and in starter cultures

Volatile fatty acids	Volatile fatty acid concentrations, μg per 100 g						
	Sterilized base milk	*S. th.* 13a	*S. th.* 15a	*L. b.* 2–11	*L. b.* 1–9	*S. th.* 13a + *L. b.* 2–11	*S. th.* 15a + *L. b.* 1–9
Acetic	460.0	960.0	780.0	1120.0	1310.0	1720.0	1880.0
Propionic	traces	60.0	90.0	traces	traces	traces	traces
Butynic	500.0	680.0	750.0	860.0	910.0	890.0	1080.0
Isovaleric	traces	30.0	50.0	traces	110.0	80.0	130.0
Valeric	120.0	240.0	180.0	290.0	260.0	340.0	320.0
Caproic	360.0	540.0	740.0	680.0	800.0	780.0	950.0
Caprylic	200.0	230.0	390.0	280.0	420.0	390.0	550.0
Capric	170.0	220.0	270.0	190.0	250.0	260.0	380.0

in the activity for the various strains [11]; esterases in both yogurt bacteria were also reported [17]. It seems that most volatile fatty acids do not originate from milk fat and their most important precursors are amino acids. It could be that the higher concentrations of individual fatty acids established in the *L. bulgaricus* 2–11 and *L. bulgaricus* 1–9 cultures are related to their proteolytic activity, which is higher than that of *S. thermophilus* 13a and *S. thermophilus* 15a. The concentrations of free amino acids in the 22-h cooled *L. bulgaricus* 2–11 and *L. bulgaricus* 1–9 cultures were 73.50 mg per 100 g and 46.50 mg per 100 g, respectively, while in the *S. thermophilus* 13a and *S. thermophilus* 15a cultures they were 2.92 mg per 100 g and 2.94 mg per 100 g, respectively. *L. bulgaricus* 2–11 and *L. bulgaricus* 1–9 had comparatively high producing ability in relation to acetic, oleic and caproic acids. The producing ability of *S. thermophilus* 13a and *S. thermophilus* 15a did not significantly differ from that of *L. bulgaricus* 2–11 and *L. bulgaricus* 1–9 as regards isovaleric, valeric, caprylic and capric acids. Because of their strong aroma and flavour, the determined volatile fatty acids C_2–C_{10} take part in the formation of the specific flavour-aromatic properties of the starter cultures and Bulgarian yogurt.

Therefore, for the formation of starter cultures, it is very important to select *L. bulgaricus* strains for their aroma-forming ability before mix-cultivating them with thermophilic streptococci, which have been selected for the same attribute.

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