# INTENSIFICATION OF THE SYNTHESIS OF FLAVOUR COMPOUNDS IN YOGURT BY MILK ENRICHMENT WITH THEIR PRECURSORS

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Key words: yogurt, acetaldehyde, flavour and aroma compounds, threonine

The aim of the present study was to test the possibility of enhancing the synthesis of flavor compounds with a traditional yogurt culture and a culture containing microflora composed of: *S. thermophilus* : *L. delbrueckii ssp. bulgaricus* : *Bifidobacterium* (TBB yogurt) and *Str. thermophilus* : *L. acidophilus* : *Bifidobacterium* (TAB yogurt). Milk was enriched with lactose (10 g/L), glucose (0.7 g/L), sodium proteinate (25 g/L), sodium citrate (3 g/L), a mixture of citric acid (1 g/L) and sodium citrate (3 g/L), and threonine (1 and 3 g/L). The following determinations were made in fresh yogurts (ripened for 24 h) and yogurts stored for 7 and 14 days: active acidity, lactic acid – according to Lunder, acetaldehyde, diacetyl, acetoin, ethanol, and volatile free fatty acids by gas chromatography. All products were evaluated organoleptically.

It was found that from among the substances whose effects on the synthesis of flavor compounds were studied in this experiment particular attention should be paid to threonine. The addition of this amino acid significantly affected the rate of acetaldehyde formation (an increase by 10.24–22.56 mg/L) in all kinds of yogurt. The modification of milk composition had a profound influence on the levels (increase or decrease) of all flavor compounds in yogurt samples, as well as on their sensoric attractiveness.

#### INTRODUCTION

The considerable increase in demand for fermented milks noted in recent years has resulted, to a great extent, from consumer awareness of their beneficial effects. However, fermented milks are also highly valued for their unique taste and aroma, which contributed to their growing popularity as well [Saint-Eve *et al.*, 2004].

The flavour and aroma of yogurt are affected by all milk components, products of their thermal degradation, and compounds formed as a result of enzymatic changes caused by homofermentative yogurt bacteria and heterofermentative bifidobacteria. The flavor attributes of yogurt depend on the concentrations of lactic acid and carbonyl compounds, mainly acetaldehyde [Raic & Kurmann, 1978; Kneifel *et al.*, 1992; Tamime & Robinson, 1999; Gueimonde *et al.*, 2003].

The source of flavor compounds in yogurt are milk components (lactose, milk fat, proteins, citrates) and products of their enzymatic degradation. The levels of these compounds are related primarily to the specific properties of bacteria used for yogurt production. However, it should be kept in mid that other key factors are the quality and kind of milk, heat treatment intensity, the content of fat and dry matter, the method and parameters of incubation, as well as the time and conditions of storage [Rašic & Kurmann, 1978; Beshkova *et al.*, 1998; Tamime & Robinson, 1999; Żbikowski, 1997]. The content of flavour compounds in natural yogurt may be controlled, to a certain degree, by modification of the factors that affect it. It seems that milk enrichment with substances which turn into flavor compounds *via* enzymatic changes may affect their levels in the final products. Thus, the aim of the present study was to determine the possibility of enhancing the synthesis of flavor compounds with yogurt cultures, following milk enrichment with their precursors.

#### MATERIALS AND METHODS

The experimental materials comprised plain yogurts (control samples) and yogurts containing the following substances (analytical grade): lactose – 10 g/L, glucose – 7 g/L, sodium proteinate – 25 g/L, introduced into milk prior to pasteurisation, and sodium citrate – 3g/L, a mixture of citric acid – 1 g/L and sodium citrate – 3 g/L, and threonine – 1 and 3 g/L, added after pasteurisation. The substances were purchased from POCH Gliwice (lactose, glucose, sodium citrate, citric acid), MERCK (threonine) and the District Dairy Cooperative in Góra Śląska (sodium proteinate). The experimental products were obtained from fresh milk supplied to the Chair of Dairy Science and Quality Management by the Dairy Plant in Olsztyn. The fat content of milk was normalised to 2%, and dry matter content was normalised to 14% with skim milk powder. Normalised milk

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was heated to 65°C, homogenised at 20 MPa, pasteurised at 95°C for 5 min and cooled to inoculation temperature. Traditional yogurt cultures and yogurt cultures containing microflora composed of: Streptococcus thermophilus (strains: TKM3, cz, CH-1-29) : Lactobacillus delbrueckii subsp. bulgaricus (strains: 168, 259, 171) at a ratio of 1:1; S. thermophilus : L. delbrueckii subsp. bulgaricus : Bifidobacterium ssp. (strains: J, 6455, 62) at a ratio of 2:1:2, (TBB yogurt) and S. thermophilus : Lactobacillus acidophilus (strains: cz1, 343, 1nd1) : Bifidobacterium ssp. at a ratio of 2:1:2. (TAB yogurt), from the collection of Danisco Biolacta, were used in the experiment. Milk was inoculated (3% of the starter) and incubated at 37 or 43°C, depending on the kind of culture used. Prior to inoculation, the cultures were passaged once in milk reconstituted from skim milk powder, and sterilised at 121°C for 10 min. Acidification was carried out until pH 4.4±0.2 was reached, *i.e.* for 4 to 6 hours. The samples were cooled, ripened for 24 h and stored at 5°C±1°C for 7 and 14 days.

The following determinations were made in the experimental materials: active acidity, lactic acid – according to Lunder [1972], acetaldehyde, diacetyl, acetoin, and ethanol – by gas chromatography following steam distillation of 100 mL of the sample, to obtain 25 mL of the distillate. Volatile flavor compounds were separated using PYE Unicam GCV with a flame-ionisation detector (FID) and a Philips recorder, at a tape feed rate of 10 mm/min. A glass column (1.5 m in length, 4 mm i.d.) was used. The solid support was a Diatomit CQ 100/120 mesh, the liquid phase was argon with a flow rate of 40 mL/min. The detector temperature was  $250^{\circ}$ C, the column temperature –  $100^{\circ}$ C, the evaporator temperature –  $150^{\circ}$ C. The calculations were performed based on a DP 68 PYE Unicam integrator.

The levels of volatile free fatty acids (VFFA) were determined by gas chromatography. The procedure was preceded by VFFA extraction and formation of soaps. VFFA were separated using a PYE Unicam GCV apparatus with a flame-ionisation detector (FID). A glass column (1.5 m in length, 4 mm i.d.) was used. The solid support was a Chromosorb WIAWI DMSC 60/80 mesh, the liquid phase was 10% DEGS+2% H<sub>3</sub>PO<sub>4</sub>, and the mobile phase – argon with a flow rate of 40 mL/min. The detector temperature was 270°C, the column temperature - 142°C, the injector temperature - 225°C. The quantities of particular VFFA were determined using an internal standard, i.e. pelargonic acid (C9:0). All products were evaluated organoleptically on a 5-point scale for each quality attribute, with the following coefficients of importance: 0.15 for appearance and consistency, 0.05 for colour, 0.35 for taste, and 0.3 for aroma.

The experiment was performed in three replications, and the results were analysed statistically using Stat 1 software. Statistical differences between the levels of particular flavor compounds in the control samples and in the samples with additives were verified at a significance level of 0.01 and 0.05.

## **RESULTS AND DISCUSSION**

The main flavoring agent in yogurt is lactic acid, which contributes to its sour, refreshing taste. Milk enrichment

Additive	Storage	pH	Lactic acid	Diacetyl	Acetoin	Ethanol	VFFA	Sensoric evaluation
	time (days)		(%)	(mg/L)	(mg/L)	(mg/L)	(mg/100g)	(points)
Control sample	1	4.45	0.86	3.90	6.20	8.20	6.54	4.20
	7	4.10	10.98	3.44	4.68	9.70	6.30	3.90
	14	3.99	31.029	2.80	5.18	9.88	6.43	3.10
Lactose	1	4.49	0.824**	5.78**	7.98**	6.11**	6.44	4.20
	7	4.25	0.870	9.64	6.50	9.32	6.69	3.90
	14	4.12	0.968	9.54	4.64	16.28	6.31	2.75
Glucose	1	4.50	0.824**	4.50	3.08**	10.76**	6.32	3.85
	7	4.29	0.916	4.84	3.54	11.32	5.92	3.40
	14	3.95	1.039	0.92	2.36	13.32	6.03	2.90
Sodium proteinate	1	4.24	0.901**	5.37**	7.76**	8.27	6.70	4.05
	7	4.17	0.943	6.84	3.40	8.64	6.39	3.55
	14	3.89	1.045	9.02	5.61	10.02	6.88	2.75
Sodium citrate	1	4.41	0.871**	2.65**	16.06**	4.04**	6.43	4.20
	7	4.15	0.970	2.78	19.08	4.36	5.85	3.90
	14	4.10	0.988	1.40	6.96	6.18	5.99	2.75
Citric acid and sodium citrat	e 1	4.15	0.907**	7.40**	3.30**	14.25**	6.03	4.20
	7	4.00	0.926	10.00	4.70	17.05	5.64	3.55
	14	3.85	1.025	7.50	4.00	19.13	5.90	3.10
Threonine (1 g/L)	1	4.37	0.858	2.72**	2.04**	5.94**	5.99	4.20
	7	4.30	0.951	2.76	1.92	4.30	6.30	3.55
	14	4.09	1.003	1.86	2.20	7.24	6.56	2.75
Threonine (3 g/L)	1	4.38	0.847**	2.93*	2.10**	6.35**	5.58	4.55
	7	4.33	0.871	3.14	2.16	4.44	5.28	3.55
	14	4.26	0.959	6.22	2.04	10.66	5.47	2.75

TABLE 1. Changes in the levels of flavor compounds in traditional yogurt influenced by the additive and storage time.

\*\* - statistically significant differences at a level of 0.01; \* - statistically significant differences at a level of 0.05; VFFA - volatile free fatty acids

with lactose, glucose, sodium proteinate, sodium citrate, a mixture of citric acid and sodium citrate, and threonine in the amount of 1 and 3 g/L affected changes in the acidity of yogurt samples and the levels of lactic acid. The acidity of fresh control products and products with additives ranged from pH 4.39 to 4.47, and from pH 4.15 to 4.61, respectively (Tables 1–3). According to Bodyfelt et al. [1988], yogurt should be acidified to pH 3.8-4.4 for best flavor characteristics. These authors believe that common flavor defects of fermented milks result from a too low level of acidity, i.e. pH above 4.5. On the other hand, Rašic & Kurmann [1978] reported that the optimum taste of yogurt may be achieved at pH 4.0-4.4. A comparison between the results of this study and the above assumptions shows that all control samples and samples with additives had adequate acidity. A slightly too low level of acidity was recorded in TBB yogurt with glucose and sodium citrate, and TAB yogurt with threonine.

The lactic acid content of the control products and yogurts with additives ranged between 0.857 and 0.881%, and between 0.812 and 0.907%, respectively (Tables 1–3). Milk enrichment with lactose and glucose – major substrates of the reaction whose product is lactic acid – significantly reduced the concentration of lactic acid in all experimental yogurts, as compared with the control samples. During milk incubation with yogurt cultures, 15% to 30% (according to some authors even 50%) of lactose is fermented to lactic acid. The degree of lactose utilisation and the rate of lactic acid formation depend on the genus and species of bacteria, and their symbiotic effect [Rašic & Kur-

mann, 1978; Tamime & Robinson, 1999; Lourens-Hattingh & Viljoen, 2001]. The results of this experiment suggest that elevated levels of lactose and glucose in milk had no influence on the degree of their utilisation for lactic acid synthesis during acidification. A significantly higher lactic acid content, in comparison with the control samples, was observed in all yogurts containing sodium proteinate (0.881–0.901%). Sodium proteinate contributed to changes in the buffer capacity of milk, so the increased concentration of lactic acid had no negative effect on the acidifying activity of yogurt bacteria. Lactic acid content was related to the kind of substances added to milk, as well as to the composition of starters.

According to Rašic & Kurmann [1978], slightly soured yogurt contains 0.85-0.95% lactic acid, whereas soured vogurt -0.96-1.2%. Following these criteria, the majority of yogurt samples in the present experiment can be described as slightly soured, and very few as not soured enough. During 7 and 14 days of storage, the acidity of both control and experimental yogurts increased gradually. The products were stored at  $5^{\circ}C \pm 1$ , which definitely reduced the biochemical activity of starter bacteria, but did not inhibit it completely. An increase in yogurt acidity over storage is most often caused by strains of L. delbrueckii ssp. bulgaricus, whose acidifying activity is high under low acidity and temperature [Lourens-Hattingh & Viljoen, 2001]. The highest increase in lactic acid content and its highest level in 14-day samples (1.015–1.030%) were recorded in yogurts containing glucose. This indicates that the cultures analysed used glucose added to milk for lactic acid production only during storage.

Additive	Storage	pН	Lactic acid	Diacetyl	Acetoin	Ethanol	VFFA	Sensoric evaluation
	time (days)		(%)	(mg/L)	(mg/L)	(mg/L)	(mg/100g)	(points)
Control sample	1	4.47	0.857	7.62	10.86	7.31	7.18	4.05
	7	4.31	0.979	8.90	11.72	8.49	6.95	4.05
	14	4.04	1.006	10.98	6.03	10.67	6.39	3.10
Lactose	1	4.45	0.850**	8.82	6.59**	8.76*	6.27	4.05
	7	4.21	0.899	12.36	7.52	9.18	6.04	3.90
	14	4.09	0.965	12.40	6.06	9.27	6.08	3.10
Glucose	1	4.52	0.819**	8.04	8.60**	7.76	5.95	3.70
	7	4.32	0.917	11.18	6.14	8.90	6.37	3.75
	14	3.99	1.024	12.52	6.93	9.77	6.06	3.10
Sodium proteinate	1	4.43	0.881**	4.39**	7.74**	6.30	7.11	4.05
	7	4.28	0.948	7.51	5.77	7.71	7.41	3.40
	14	3.97	0.989	7.30	6.19	9.74	7.08	3.10
Sodium citrate	1	4.53	0.826**	1.78**	4.22**	3.62**	6.27	4.05
	7	4.44	0.862	2.35	4.64	4.30	6.40	3.70
	14	4.33	0.901	6.96	7.12	4.30	5.76	3.10
Citric acid and sodium citrate	e 1	4.43	0.862**	7.62	7.64**	10.60**	6.13	3.70
	7	4.32	0.939	9.46	7.48	12.41	6.39	4.05
	14	3.98	1.017	10.34	11.38	13.47	6.35	3.10
Threonine (1 g/L)	1	4.47	0.889**	2.01**	1.76**	8.96*	6.88	4.40
	7	4.15	0.907	2.42	3.18	13.06	6.53	4.05
	14	4.10	0.995	4.93	4.08	9.96	6.45	3.10
Threonine (3 g/L)	1	4.42	0.884**	2.62**	6.44**	3.92**	6.29	4.40
	7	4.23	0.901	4.20	2.58	5.52	5.63	3.70
	14	4.17	0.969	8.78	4.82	5.96	6.86	3.10

TABLE 2. Changes in the levels of flavor compounds in TBB yogurt influenced by the additive and storage time.

\*\* - statistically significant differences at a level of 0.01; \* - statistically significant differences at a level of 0.05; VFFA - volatile free fatty acids

TABLE 3. Changes in the levels of flavor	compounds in	TAB yogurt influenc	ed by the a	dditive and storage time.

Additive	Storage time (days)	pН	Lactic acid (%)	Diacetyl (mg/L)	Acetoin (mg/L)	Ethanol (mg/L)	VFFA (mg/100g)	Sensoric evaluation (points)
Control sample	1	4.39	0.881	7.12	16.26	9.01	7.00	4.05
	7	4.30	0.915	8.43	6.05	9.33	6.75	4.05
	14	4.12	1.006	10.08	5.10	9.97	6.84	3.25
Lactose	1	4.41	0.861**	7.14	6.45**	7.94	6.23	3.70
	7	4.30	0.933	9.42	5.08	11.71	6.06	3.70
	14	4.07	0.989	11.79	8.49	13.50	6.16	3.10
Glucose	1	4.33	0.853**	9.40*	4.40**	12.78**	5.79	3.70
	7	4.11	0.947	11.96	5.80	15.99	5.87	4.05
	14	3.98	1.015	13.58	3.56	15.72	5.96	3.25
Sodium proteinate	1	4.38	0.889**	8.32	7.08**	9.52	7.15	4.05
	7	4.19	0.969	10.46	9.14	9.94	6.30	4.05
	14	3.95	1.006	12.52	9.82	10.56	6.88	3.10
Sodium citrate	1	4.42	0.838**	9.26*	5.74**	10.56	6.91	4.05
	7	4.18	0.857	6.86	5.18	6.02	6.34	3.70
	14	4.07	0.901	2.78	5.58	4.76	6.72	3.25
Citric acid and sodium citrate	e 1	4.43	0.857**	10.94**	6.18**	11.14*	6.16	4.05
	7	4.31	0.912	12.40	7.66	13.04	6.52	3.70
	14	4.01	1.004	13.02	7.80	14.68	6.27	3.10
Threonine (1 g/L)	1	4.61	0.841**	6.20	3.86**	9.28	7.04	4.40
	7	4.52	0.967	5.30	2.92	21.54	6.17	4.05
	14	4.37	0.991	2.95	7.06	10.94	5.72	3.25
Threonine (3 g/L)	1	4.53	0.855**	3.30**	2.58**	4.62**	5.96	4.40
	7	4.44	0.892	4.26	2.66	4.58	5.55	3.70
	14	4.27	0.900	12.42	2.78	8.24	5.34	3.25

\*\* - statistically significant differences at a level of 0.01; \* - statistically significant differences at a level of 0.05; VFFA - volatile free fatty acids

The main volatile compound affecting the flavour of yogurt is acetaldehyde. The concentration of acetaldehyde in the control samples was as follows: 14.88 mg/L in plain yogurt, 10.10 mg/L in TBB yogurt, and 16.06 mg/L in TAB yogurt (Figures 1–3). The level of acetaldehyde required to develop the desirable flavor of yogurt has been widely discussed among experts, and is believed to range from 8 to 50 mg/L [Rašic & Kurmann, 1978; Kneifel *et al.*, 1992; Imhof & Bosset, 1994; Tamime & Robinson, 1999]. The most common level, cited by Bottazzi *et al.* [1973], is 10–15 mg/L. It follows that the acetaldehyde content of yogurts in this study can be considered satisfactory.

Among the additives modifying the acetaldehyde content of yogurt particular attention should be paid to threo-



FIGURE 1. Changes in the level of acetaldehyde in traditional yogurt influenced by the additive and storage time.

nine. Milk enrichment with this amino acid, in the amount of 1 and 3 g/L, caused a significant increase in acetaldehyde content, by 16.34 mg/L and 12.94 mg/L in traditional yogurt, by 10.24 mg/L and 22.56 mg/L in TBB yogurt, and by 15.90 mg/L and 18.64 mg/L in TAB yogurt, compared with the control samples. These results are consistent with those obtained by other authors [Marshall & Cole, 1983; Rysstad & Abrahamsen, 1987; Gomes & Malcata, 1999; van Kranenburg *et al.*, 2002], who stress the role of threonine as a key substrate in the reaction of acetaldehyde formation. Threonine is metabolised to acetaldehyde and glycine in the presence of threonine aldolase, which is active in both lactic acid rods and streptococci [Marshall & Cole, 1983; Ott *et al.*, 2000].



FIGURE 2. Changes in the level of acetaldehyde in TBB yogurt influenced by the additive and storage time.



FIGURE 3. Changes in the level of acetaldehyde in TAB yogurt influenced by the additive and storage time.

As a result of the reaction catalysed by alcohol dehydrogenase, acetaldehyde is reduced to ethanol, which decreases the quality and intensity of yogurt flavor [Rysstad & Abrahamsen, 1987; Engels & Visser, 1996; de Vos & Hugenholtz, 2004]. In the present study, an elevated level of ethanol, accompanied by a lower acetaldehyde content, in comparison with the control samples, was recorded in all yogurts with glucose or a mixture of citric acid and sodium citrate. The highest decrease in acetaldehyde, by 9.5 mg/L, and the highest increase in ethanol, by 3.77 mg/L, were observed in the samples containing L. acidophilus bacteria. The same pattern of changes was followed in the majority of the remaining samples. The only exceptions were the samples with threonine, and TBB and TAB samples containing 3 g/L of this amino acid. These products had the highest concentration of acetaldehyde and a significantly lower, compared with the control samples, concentration of ethanol. This could be caused by the inhibition of the activity of alcohol dehydrogenase by the substrate. The concentration of ethanol in traditional yogurt and in TAB yogurt containing glucose, sodium proteinate, sodium citrate and 1 g/L of threonine was higher than in TBB yogurt. This could be a consequence of the presence of L acidophilus bacteria in this product. According to Marshall & Cole [1983], the enzymatic system of these strains includes a particularly active alcohol dehydrogenase. During the entire storage period, the concentration of ethanol increased gradually in the majority of samples, regardless of changes in the level of acetaldehyde.

A number of authors [Rašic & Kurmann, 1978; Tamime & Robinson, 1999, de Vos & Hugenholtz, 2004] share the opinion that acetaldehyde is produced primarily as a result of lactose conversion. However, the addition of this sugar to milk caused a significant increase (by 2.74 mg/L) in acetaldehyde content, as compared with the control samples, in TBB yogurt only. In the glucose-enriched samples the level of this compound was significantly lower. During 7- and 14-day storage the concentration of acetaldehyde decreased in the control samples and in the majority of samples with additives. Only in yogurt containing 3 g/L of threonine did the level of acetaldehyde increase over the entire storage period.

An important flavor compound in yogurt is diacetyl, formed as a result of transformations of citrates and/or lactose [van Kranenburg *et al.*, 2002; de Vos & Hugenholtz, 2004]. Diacetyl is always accompanied by acetoin, which is a non-volatile, odour-free compound that does not directly affect the flavour of yogurts. Acetoin may be formed as a result of diacetyl conversion catalysed by diacetyl reductase, or decarboxylation of  $\alpha$ -acetolactic acid, depleting the medium of this compound and thus affecting the sensory quality of yogurts [Tamime & Robinson, 1999; de Vos & Hugenholtz, 2004].

Yogurts enriched with lactose and glucose had a higher level of diacetyl (4.50-9.40 mg/L) than the control samples (3.90-7.62 mg/L), but this difference was statistically significant only in the case of traditional yogurt with lactose and TAB yogurt with glucose (Tables 1–3). The level of acetoin was also elevated in these samples. A significant decrease in diacetyl and acetoin was observed in the samples containing 1 or 3 g/L of threonine. The addition of citrate to milk, considered the main source of diacetyl, caused a significant increase in the quantity of this compound in traditional yogurt and TAB yogurt with a mixture of citric acid and its salts, to the highest values, i.e. 7.40 mg/L and 10.94 mg/L, respectively. The lowest level of diacetyl (1.78 mg/L) was recorded in TBB yogurt containing sodium citrate. These results do not, however, explicitly confirm previous reports according to which only pyruvate formed by citrate conversion is used for diacetyl biosynthesis, and bifidobacteria are the only yogurt bacteria able to utilise these compounds [de Vos & Hugenholtz, 2004, Rysstad & Abrahamsen, 1987]. During storage, the concentrations of diacetyl and acetoin decreased in the control samples of traditional yogurt, whereas in the other samples the level of diacetyl increased and the level of acetoin decreased. Diacetyl content increased in the majority of samples with additives, whereas changes in acetoin content were rather irregular.

Another group of compounds responsible for the flavour of yogurt are volatile free fatty acids. Their concentration in the control samples of natural yogurt amounted to 6.54 mg/100 g of the product, and was lower than in the other samples (7.00-7.18 mg/100 g of the product) (Tables 1-3). Milk enrichment with additives altered the levels of VFFA in the final products to a slight degree. However, the highest concentrations of VFFA were recorded in all yogurts containing sodium proteinate (7.11–7.15 mg/100 g of the product). These results agree with the opinion shared by some authors that VFFA may be produced in consequence of lactose conversion and during oxidative deamination and transamination of amino acids [Beshkova et al., 1998; Tamime & Robinson, 1999; Kranenburg et al., 2002]. Changes in the levels of volatile free fatty acids observed in all samples over the entire storage period were minor.

Among the additives used in the study only threonine added to milk improved the quality of yogurt with modified microflora and traditional yogurt with a greater amount of this amino acid. This was confirmed by the fact that these products got a higher (by 0.35) score during a sensoric evaluation (Tables 1–3). Their taste improved considerably. It seems that the increase in acetaldehyde content was the main reason for the high score of these samples in the organoleptic evaluation.

Glucose had a negative effect on the organolpetic properties of yogurts. The taste of these samples deteriorated due to a significant decrease in acetaldehyde content, accompanied by an increase in ethanol content. Traditional yogurts with sodium proteinate and TAB yogurt with lactose were also characterised by a slightly lower quality. Yogurts enriched with sodium proteinate had consistency defects – their texture was too firm and lumpy. The quality of both control samples and yogurts with additives deteriorated during the storage.

### CONCLUSIONS

From among the substances whose effects on the synthesis of flavour compounds were studied in this experiment particular attention should be paid to threonine. The addition of this amino acid in the amount of 1 and 3 g/L significantly affected the rate of acetaldehyde formation in traditional yogurt (an increase by 87–110%) and in yogurt with modified microflora (an increase by 99–223%).

Milk enrichment with the additives analysed had a profound influence on the levels of particular flavour compounds in all yogurts. There were statistically significant differences in the levels of these compounds between the control and enriched samples. However, in many cases the addition of precursors of flavouring substances to milk inhibited their formation by yogurt cultures. In addition, changes in the amounts of flavour compounds not always positively affected the sensory properties of the final products.

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# INTENSYFIKACJA SYNTEZY ZWIĄZKÓW SMAKOWO–ZAPACHOWYCH W JOGURCIE POPRZEZ WZBOGACENIE MLEKA W ICH PREKURSORY

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Podjęte badania miały na celu określenie możliwości intensyfikacji syntezy związków smakowo-zapachowych przez kultury jogurtowe: tradycyjną oraz z dodatkową mikroflorą o następującym składzie: *S. thermophilus* : *L. delbrueckii ssp. bulgaricus* : *Bifidobacterium* (jogurt TBB) i *Str. thermophilus* : *L. acidophilus* : *Bifidobacterium* (jogurt TAB). Mleko wzbogacano w laktozę (10 g/L), glukozę (0.7 g/L), białczan sodu (25 g/L), cytrynian sodu (3 g/L), mieszaninę kwasu cytrynowego (1 g/L) i cytrynianu sodu (3 g/L) oraz treoninę (1 i 3 g/L). W świeżych (po 24 godz. dojrzewania) wyrobach jogurtu oraz przechowywanych przez 7 i 14 dni oznaczano: kwasowość czynną, zawartość kwasu mlekowego wg Lundera; aldehydu octowego, diacetylu, acetoiny, alkoholu etylowego, lotnych kwasów tłuszczowych metodą chromatografii gazowej. Wszystkie wyroby oceniano organoleptycznie.

Na podstawie uzyskanych wyników stwierdzono, że z substancji, których wpływ na syntezę związków smakowo-zapachowych badano w doświadczeniu, na wyróżnienie zasługuje treonina. Dodatek tego aminokwasu wpłynął istotnie na intensywność tworzenia aldehydu octowego we wszystkich rodzajach jogurtu (wzrost zawartości o 10.24–22.56 mg/L) (rys. 1–3). Modyfikacja składu mleka miała wyraźny wpływ na zawartość (wzrost lub obniżenie) wszystkich związków smakowo-zapachowych w próbkach jogurtów a tym samym na ich jakość organoleptyczną (tab. 1-3).