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THE CONTENT OF SENSORY ACTIVE COMPOUNDS AND FLAVOUR OF SEVERAL TYPES OF YOGURTS

E. Vítová, L. Babák, R. Mokáňová, E. Hýsková, J. Zemanová

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Abstract

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The aim of this work was to identify and quantify several sensory active compounds in various types of yogurts using gas chromatography and simultaneously to judge their influence on flavour of yogurts using sensory analysis. In total 4 types of white and 10 types of flavoured yogurts (creamy and low-fat) with various flavourings, produced in Dairy Valašské Meziříčí, Ltd., were analysed.
The highest content of sensory active compounds ($P < 0.05$) was found in strawberry yogurts, with high amount of ethyl butyrate. Excepting ethanol no significant differences ($P < 0.05$) were found between low-fat and creamy varieties. The total content of sensory active compounds in white yogurts was significantly ($P < 0.05$) lower than in flavoured fruit types. The highest content was in low-fat and lowest in white bio yoghurts.
Flavour of yogurts was evaluated sensorially using scale and ranking test. All creamy yogurt varieties were evaluated as significantly ($P < 0.05$) more tasty than low-fat ones. Similarly in case of white yogurts creamy yogurts were evaluated as the most tasty and low-fat ones as the worst. Bio yogurts were evaluated equally tasty as classic yogurts with the same fat content.

yogurt, flavour, sensory active compounds, GC, SPME, sensory analysis

Yogurts are the most widespread fermented dairy products worldwide. They are appreciated for nutritive and dietetic properties, however, the organoleptic properties, i.e. appearance, colour, texture, taste and aroma, formed by so called sensory active compounds (SAC), are the most important for consumers. SAC are all the odorous and taste compounds that form flavour of foods. Fermentation process and thermal treatment of foods are the main processes where these compounds are created from their precursors (Kailasapathy, 2006; Nongonierma *et al.*, 2006). Several hydrocarbons, oxygen derivatives (aldehydes, alcohols, ketones, ethers, fatty acids and their esters), nitrogen (amines) or sulphur (thiols) compounds are included in SAC (Gardini *et al.*, 1999). The understanding of conditions and regularities of SAC creation and their influence on sensory perception of flavour is necessary for production of high-quality and tasty products.

The Czech legislation, Directive 77/2003 Coll. (2003), amended by 124/2004 Coll. (2004), defines yogurt as a fermented dairy product acquired by fermentation of milk, cream, buttermilk or their mixture, using microorganisms *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*. Technological process of yogurt production consists of several steps. The fermentation phase, where not only characteristic taste and aroma, but also structure and consistence of yogurts are developed, is the most important. Taste and aroma active compounds are products of metabolism of used cultures. They arise by enzymatic decomposition of lactose, proteins and fat in milk (Beshkova *et al.*, 1998). Several SAC come directly from milk used for production (aldehydes, ketones, alcohols).

The anaerobic transformation of saccharides to lactic acid is the basic biochemical pathway during yogurt production. The produced lactic acid imparts fine refreshing acid taste to these products and extends their durability. It also precipitates milk proteins, which are then easier to digest and improves utilization of calcium, phosphorus and iron. The part of lactic acid comes to colon, where acidifies environment and prevents development of putrefactive microflora (Roginski *et al.*, 2003). The proteolytic activity of lactic acid bacteria changes physico-chemical properties of casein, which influences rigidity, texture and viscosity of yogurt coagulate. Arisen peptides and free amino acids are precursors for enzymic reactions producing taste compounds. However, too intensive proteolysis can cause taste defects and creation of soft yogurt coagulate. Similarly SAC can arise by lipolytic activity. Released fatty acids (FA) are precursors for methyl ketones, alcohols, lactones and esters, which impart characteristic flavour to yogurts (Roginski *et al.*, 2003).

Many authors were interested in flavour and SAC content of various types of yogurts. Although many SAC were identified in yogurts until now, only a small part of them probably influences the final aroma of yogurt, primarily these which are present in high concentrations (Beshkova *et al.*, 1998). Most of authors agree, that acetaldehyde (10–20 mg.kg^{-1}), acetic acid (20–30 mg.kg^{-1}), sufficient acidity caused by lactic acid and free volatile saturated FA are necessary for typical flavour of yogurt (Georgala *et al.*, 1995; Ott *et al.*, 2000b; Mei *et al.*, 2004).

The aim of this work was to identify and quantify SAC in various types of white and flavoured yogurts using gas chromatography-mass spectrometry (GC-MS) with SPME (solid-phase microextraction) extraction and to judge their influence on flavour of yogurts using sensory analysis.

MATERIALS AND METHODS

Samples

In total, 14 kinds of stirred yogurts were used for analysis. Yogurts were produced in Dairy Valašské Meziříčí, Ltd., their flavourings and fat contents are given in Tab. I. Three batches of every yogurt type was taken, every sample was analysed three times. Prior to analysis, each yogurt was mixed by spoon, 1 g of mixed sample was placed into vial for SPME extraction of SAC and about 20 g of sample in glass containers were used for sensory analysis.

SPME-GC-MS conditions

SPME fiber CAR™/PDMS 85 µm (Supelco). Sample weight 1 g, extraction temperature 35 °C, equilibrium time 30 min., extraction time 20 min., desorption temperature 250 °C, desorption time 5 min.

Gas chromatograph TRACE™ GC (ThermoQuest, I), capillary column DB-WAX (30m × 0,32 mm × 0,5 µm). Injector 250 °C, splitless desorption 5 min., carrier gas N_2 0,9 ml.min^{-1}, flame ionization detector (FID) at 220 °C, H_2 35 ml.min^{-1}, air 350 ml.min^{-1}, make up N_2 30 ml.min^{-1}. The oven temperature was 40 °C for 1 min, 40–200 °C at 5 °C/min, 200 °C for 7 min.

GC-MS analyses were made on GC 8000 (Carlo Erba, I) with MS TRIO 1000 (Fisons Instruments, USA). Carrier gas He, GC column and conditions were the same as described above.

The validation parameters of SPME-GC-MS method were published previously (Vítová *et al.*, 2006; Vítová *et al.*, 2007).

Sensory analysis

The sensorial characteristics of yogurt samples were evaluated by 40 basically trained assessors. Their evaluation can be considered as adequate to common consumers.

I: *The overview of yogurt samples analysed*

Yogurt type			flavouring	Fat (% w/w)
I.	Creamy	Ia	strawberry	8.0
		Iav	strawberry-vanilla	8.0
		Ib	raspberry	8.0
		Ibv	raspberry-vanilla	8.0
		Ic	sour cherry	8.0
		Icv	sour cherry-vanilla	8.0
		Id	peach	8.0
II.	Low-fat	IIa	strawberry	0.1
		IIb	raspberry	0.1
		IIc	peach	0.1
III.	White	IIIa	creamy	10.0
		IIIb	middle-fat	3.0
		IIIc	middle-fat bio	3.0
		IIId	low-fat	0.1

The flavour of yogurts was evaluated using five point ordinal scale (1 – excellent, 2 – very good, 3 – good, 4 – acceptable, 5 – unacceptable), and then using ranking test (the most tasty → the least tasty sample) following ISO 8587 (2006).

The specialized sensory test room according to the ISO 8589 (2007) was used.

Statistical evaluation

All results were evaluated using the variation statistics (ANOVA) to Snedecor and Cochran (1967) using the statistical package Unistat, v. 5.5. The results are expressed as mean ± SD (n = 9).

RESULTS AND DISCUSSION

Identification and quantification of sensory active compounds

SAC in yogurt samples were extracted by SPME, identified by GC-MS, confirmed and quantified using standards by GC-FID. Standards were purchased from Sigma-Aldrich (Deisenhofen, Germany) and Merck (Darmstadt, Germany).

In total 30 various organic compounds belonging to five chemical groups were identified in yogurt samples. Ten alcohols were in the first group: ethanol, 2-methyl-2-propanol, 2-butanol, 3-methylbutan-1-ol, pentan-2-ol, pentan-1-ol, hexan-1-ol, heptan-2-ol, octanol and decan-1-ol, two aldehydes in the second group: acetaldehyde and benzaldehyde, six ketones in the third group: acetone, 2-butanone, heptan-2-one, nonan-2-one, biacetyl and acetoin, six organic acids in the fourth group: acetic, propionic, butyric, caprylic, lactic and 2-methylpropionic and six esters in the fifth group: methyl acetate, ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate and phenyl acetate.

Many authors dealt with the assessment of SAC in yogurts, e.g. Gardini *et al.* (1999), Lubbers *et al.* (2004), Kaminarides *et al.* (2007), Ligor *et al.* (2008). Interestingly, most of published works analyses strawberry yogurts, which is probably the most favourite flavour worldwide, and then yogurts and yogurt products without flavouring. Ligor *et al.* (2008) consider biacetyl, acetoin, lactic acid and acetaldehyde to be the significant components of yogurt aroma. Kaminarides *et al.* (2007) consider acetic acid, acetaldehyde, acetone, biacetyl, 2-butanone, acetoin and 3-methyl-2-butanone to be the main SAC in yogurt. Gardini *et al.* (1999) isolated many SAC from yogurt type products; acetaldehyde, acetic acid, acetone, biacetyl and 2-butanone were the most abundant. Moreover, taste quality of yogurts depends also on relationships among single volatile compounds. The role of biacetyl in yogurt aroma is still controversial. Several authors claim, that biacetyl significantly contributes to aroma only if concentration of acetaldehyde is low, the others consider it to be important component of yogurt aroma (Imhof *et al.*, 1995; Beshkova *et al.*, 1998). Hernaindez *et al.* (1995) rate nonvolatile fatty acids (e.g. lactic, pyruvic), volatile acids (e.g. formic, acetic, propionic), carbonyl compounds (e.g. acetaldehyde, acetone, acetoin, biacetyl) and heterogeneous group of compounds formed during thermal degradation of proteins, fats and lactose, among main aroma compounds of yogurt. Biacetyl, lactic acid and acetaldehyde influence the final taste and their concentration determines the quality and flavour for consumers. Imhof *et al.* (1995) consider only biacetyl, pentane-2,3-dione, dimethylsulphide and benzaldehyde to be important for yogurt flavour. To summarize above written results, acetaldehyde, acetone, ethyl acetate, biacetyl, ethanol, acetoin, acetic, butyric and lactic acids probably take part in typical yogurt aroma as the most important ones. All these compounds were identified in our samples; ethanol, acetaldehyde, acetone, acetoin and acetic acid in concentration >10 $mg.kg^{-1}$. The comparison of the content of these chosen compounds in various yogurts is given in Fig. 1.

Single flavourings were compared in fruit flavoured yogurts. The highest total content of SAC ($P < 0.05$) was in strawberry yogurts: low-fat (1543.5 ± 9.17 $mg.kg^{-1}$), creamy (837.4 ± 1.62 $mg.kg^{-1}$) as well as with vanilla (1140.3 ± 5.43 $mg.kg^{-1}$). The high amount of ethyl butyrate (strawberry 659.9 ± 9.78 $mg.kg^{-1}$, strawberry-vanilla 339.2 ± 9.89 $mg.kg^{-1}$, strawberry low-fat 1386.2 ± 7.34 $mg.kg^{-1}$) was found here. It is probably a typical part of strawberry aroma. This confirms the results of Decourcelle *et al.* (2004) and Lubbers *et al.* (2004). No similar characteristic component was identified in other flavourings.

Substantially higher content of SAC ($P < 0.05$) was determined in every fruit variety combined with vanilla. Especially the presence of ethanol, acetaldehyde and acetone contributed to this fact (see Fig. 1). Not even here some characteristic component typical for vanilla aroma was identified.

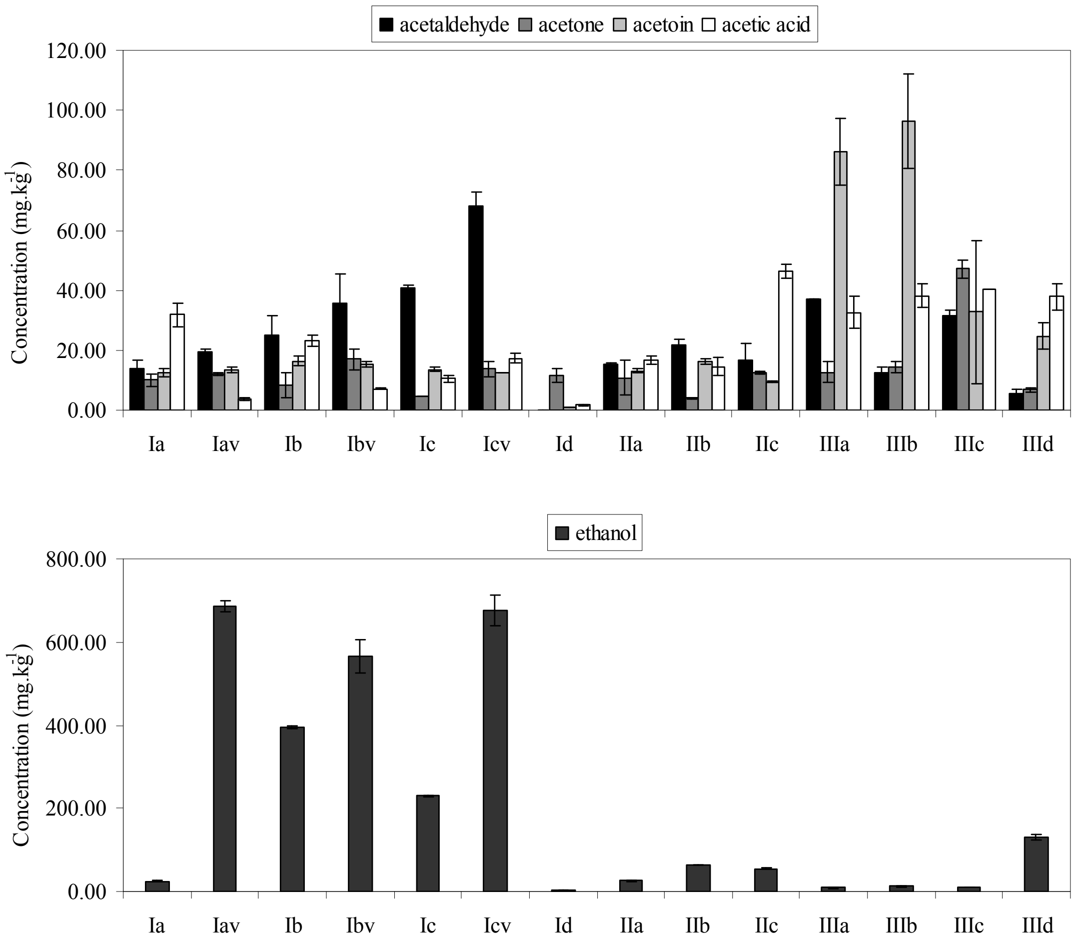
No statistically significant differences between low-fat and creamy varieties in total content and single chemical groups of SAC were found (see Tab. II). Only the content of ethanol was markedly higher in creamy varieties (see Fig. 1). The higher fat content contributes to better flavour of yogurts (which is apparent from sensory evaluation), primarily by pleasant perception of fat on tongue.

Varieties with various fat content were compared in white yogurts. Expectably the content of SAC groups differed slightly (see Tab. II). This could be caused, to a certain extent, by different fattiness, but also by using various starter cultures. Different starter cultures are used for production of low-fat yogurts because of compensation of low fat content. Milk from eco farms, whose composition can be different from composition of standard milk, is used for production of bio yogurts. The highest content of SAC was in low-fat (267.1 ± 1.57 $mg.kg^{-1}$) and lowest ones in white middle-fat bio (189.9 ± 4.64 $mg.kg^{-1}$) yoghurts. However, the total content of SAC was significantly ($P < 0.05$) lower than in flavoured fruit types, so we can conclude that substantial part of yogurt aroma is created by contribution

II: *Total content of single chemical groups of sensory active compounds in yogurt samples (mg.kg⁻¹)*

	Flavoured creamy yogurts						
	Ia	**Iav**	**Ib**	**Ibv**	**Ic**	**Icv**	**Id**
alcohols	54.7 ± 2.06	701.1 ± 1.08	409.4 ± 2.21	570.6 ± 5.27	235.5 ± 3.45	685.3 ± 2.44	12.7 ± 3.14
aldehydes	13.7 ± 0.21	19.2 ± 2.74	25.3 ± 1.48	35.6 ± 0.68	52.4 ± 1.23	77.8 ± 1.32	0.4 ± 0.01
ketones	26.6 ± 1.07	31.3 ± 4.37	29.3 ± 0.85	37.1 ± 2.04	20.5 ± 0.99	31.2 ± 1.98	19.3 ± 1.28
organic acids	37.6 ± 0.15	16.7 ± 0.58	33.7 ± 1.76	15.2 ± 1.36	18.7 ± 1.46	22.0 ± 1.65	8.8 ± 0.48
esters	705.2 ± 1.24	371.8 ± 2.34	78.4 ± 3.46	7.8 ± 0.05	4.9 ± 0.02	7.9 ± 0.02	4.9 ± 0.03
	Flavoured low-fat yogurts			**White yogurts**			
	IIa	**IIb**	**IIc**	**IIIa**	**IIIb**	**IIIc**	**IIId**
alcohols	63.8 ± 0.43	98.5 ± 1.66	215.8 ± 3.25	14.7 ± 1.06	14.1 ± 0.01	14.3 ± 0.49	167.7 ± 3.25
aldehydes	15.2 ± 1.94	21.8 ± 3.01	20.4 ± 1.74	37.2 ± 1.88	12.5 ± 0.45	31.5 ± 1.03	5.5 ± 0.04
ketones	27.7 ± 2.47	25.5 ± 0.95	26.6 ± 1.32	119.3 ± 4.79	123.6 ± 1.89	93.4 ± 3.26	39.2 ± 2.11
organic acids	23.4 ± 0.26	23.5 ± 0.15	52.8 ± 2.06	40.2 ± 2.04	52.2 ± 2.03	47.9 ± 5.12	48.1 ± 0.43
esters	1413.2 ± 8.46	20.5 ± 1.78	39.9 ± 1.01	1.5 ± 0.03	2.4 ± 0.02	2.5 ± 0.02	6.3 ± 0.12

Data shown are mean ± SD; n = 9



1: *Comparison of chosen sensory active compounds in yogurt samples (mg.kg⁻¹)*
I – Flavoured creamy yogurts (fat 8% w/w)
II – Flavoured low-fat yogurts (fat < 0.1% w/w)
III – White yogurts

of various flavourings. Significantly higher content of acetic acid and acetoin, on the other hand significantly lower content of ethanol ($P < 0.05$) was found in white yogurts, compared to fruit flavoured creamy yogurts (see Fig. 1). Hence ethanol is probably the part of fruit flavourings.

Nowadays more and more yogurts in bio quality hit the market. One type of white middle-fat bio yogurts was analysed in this work. Surprisingly the SAC content was relatively low and did not differ significantly from classic middle-fat white yogurts. No significant difference was also found in their flavour sensorially. Only finding of significantly ($P < 0.05$) high content of acetone ($47.1 \pm 3.56\,mg.kg^{-1}$) (see Fig. 1) was interesting, we can assume that it comes from milk used.

Sensory analysis

Flavour of yogurts should be harmonic, balanced sweet and acid, fresh, with creamy mouthfeel. Many authors evaluated sensorially flavour of various types of yogurts, e.g. Ott *et al.* (2000a) consider acidity to be the most influencing yogurt flavour, SAC are only the minor factor. Kalhotka *et al.* (2009) observed changes of organoleptic properties of white yogurts during storage. Pohjanheimo and Sandell (2009) judged sensorially flavour of four types of yogurt products in dependence on other properties. Assessors preferred acid taste, while consumers preferred sweeter taste.

In our case single flavourings were mutually compared in flavoured yogurts and then every creamy yogurt with corresponding low-fat variety. We can conclude that no fruit yogurt flavouring was evaluated as significantly tastier than the others. Also fruit-vanilla varieties surprisingly were not tastier than fruit ones alone, although the content of SAC was higher. The choice probably depends only on taste of consumer. Nevertheless all creamy yogurt varieties were evaluated as significantly ($P < 0.05$) tastier than low-fat ones. Expectably consumers unambiguously prefer creamy yogurts. The higher fat content contributes to milder and more delicious yogurt flavour; on the other hand it increases energy value of yogurts.

Similarly in case of white yogurts creamy yogurt was evaluated as the most tasty ($P < 0.05$) and low-fat one as the worst. Bio yogurts were evaluated equally tasty as classic yogurts with the same fat content. Raw materials in bio quality, used for its production, probably do not influence organoleptic properties of final products.

SOUHRN

Obsah senzoricky aktivních látek a chutnost různých typů jogurtů

Metodami GC-MS a GC-FID byly identifikovány a kvantifikovány vybrané těkavé senzoricky aktivní látky v různých typech bílých a ochucených jogurtů. Senzorickým hodnocením byla posouzena celková chutnost jogurtů. K extrakci a zakoncentrování byla použita mikroextrakce tuhou fází. Byly analyzovány čtyři druhy bílých jogurtů a deset druhů smetanových a nízkotučných jogurtů s různými příchutěmi, vyrobených v Mlékárně Valašské Meziříčí, spol. s r.o.

Nejvyšší obsah senzoricky aktivních látek ($P < 0{,}05$) byl nalezen v jahodových jogurtech, ve vysokém množství zejména etylbutyrát. Kromě etanolu nebyly nalezeny statisticky významné rozdíly mezi nízkotučnými a smetanovými variantami. Celkový obsah senzoricky aktivních látek v bílých jogurtech byl významně ($P < 0{,}05$) nižší než v ovocných jogurtech. Nejvyšší celkový obsah senzoricky aktivních látek měly jogurty nízkotučné, nejnižší jogurty bio.

Při senzorickém hodnocení byly ve všech případech jako významně chutnější ($P < 0{,}05$) hodnoceny jogurty smetanové oproti nízkotučným. Jogurty bio byly chuťově hodnoceny stejně jako klasické jogurty se stejným obsahem tuku.

jogurt, chutnost, senzoricky aktivní látky, GC, SPME, senzorická analýza

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Address

Ing. Eva Vítová, Ph.D., Ing. Libor Babák, Ph.D., Ing. Radka Mokáňová, Ing. Eva Hýsková, Ing. Jana Zemanová, Ph.D., Ústav chemie potravin a biotechnologií, Fakulta chemická, Vysoké učení technické v Brně, Purkyňova 118, 612 00 Brno, Česká republika, e-mail: evavitova@post.cz