Aroma profiles and sensory evaluation of yogurt during storage*

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Summary

It is known that more than 100 chemical compounds have been isolated from yogurt and related milk products but only a few (acetaldehyde, ethanol, aceton, diacetyl and butanon-2) have a high impact on the desired product flavour. The concentration of these typical flavour compounds changed during storage depending on duration and temperature in depot. In this article six commercial brands of plain yogurt were stored within 10 days on two different temperatures and every two days changes in acetaldehyde, diacetyl and ethanol were established. At the same time sensory evaluation was carried out as well. Acetaldehyde and ethanol concentration was determined using aldehyde dehydrogenase and alcohol dehydrogenase method. Diacetyl was measured according to Hill's modification of colorimetric method. A five member panel, performed sensory analysis using scoring system with weighted factors in the 20-points' scale. Expert panel's ratings were regressed against concentrations of aroma compounds for both temperature levels and linear relationship among them were determined. The most pronounced relationship expressed as correlation coefficients (r) between expert panel's ratings and concentrations of individual compounds was observed with ethanol (r=0.996) in yogurt sample stored at +4°C. Coefficients of correlation were generally high (from 0.824 to -0.996) and consequently very accurately predicting equations were obtained using regression analysis.

Key words: aroma compounds, acetaldehyde, diacetyl, ethanol, sensory evaluation, correlation

Introduction

Fermented milks are accepted by the consumer because of their characteristic properties: flavour, aroma, appearance and texture. All these sensoric qualities are consequences of multiple fermentations and it is accepted that selection of the multi-strain or more often multi-species inoculum is necessary to obtain a good fermented product. Therefore, the last twenty years considerable advances have been made towards an understanding of cultured product flavours and the role of micro-organisms in the formation and degradation of these flavours.

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It is known that more than 100 chemical compounds have been isolated from yogurt but only a few (acetaldehyde, ethanol, aceton, diacetyl and butanon-2) have a high impact on the desired product flavour. Although acetaldehyde, diacetyl, acetoin, acetone and butanon-2 are all present in yogurt acetaldehyde is recognized as the main flavour component (Ulberth and Kneifel, 1992; Kneifel and Ulberth, 1992; Ulberth, 1991.).

Metabolic routes to flavour production are presented on following figures.

Figure1 Pathways of lactose catabolism (Davies and Law, 1984.)
Slika 1. Putevi razgradnje laktoze

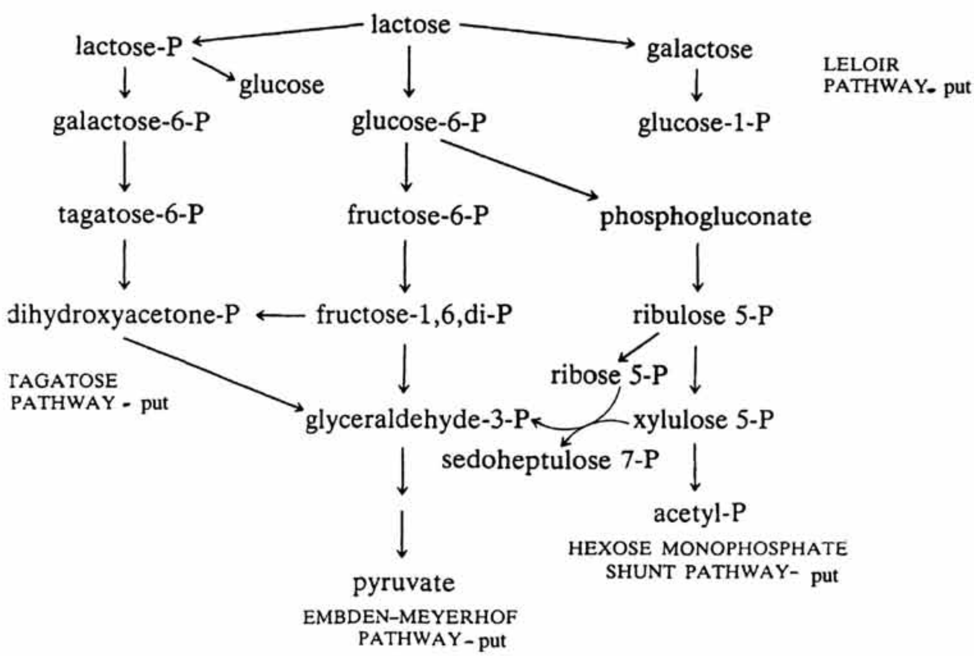

Figure 1 shows many of the relevant catabolic pathways relative to lactose breakdown, but it is important to realize that not all of the pathways are common to all of the micro-organisms fermenting milk. Thus, *Str. thermophilus* appears to use only the Embden-Meyerhof pathway (Fig. 1).

The pathways describing the fates of pyruvate are shown in Figure 2 (Davies and Law, 1984.).

Acetaldehyde has been considered to have central role in metabolism. The *Streptococcus* may form acetaldehyde from lactose via pyruvate (Fig. 2), but only trace amounts are formed via this route by *Lb. bulgaricus.*

Fig. 2. Metabolism of pyruvate
Slika 2. Metabolizam piruvata

1. pyruvate $\xrightarrow{\text{NADH} \rightarrow \text{NAD}}$ lactate

2. pyruvate → "active aldehyde" → acetyl CoA + CO_2

3. acetyl CoA + "active aldehyde" → diacetyl

4. acetyl CoA → acetaldehyde

5. acetate + ATP → acetyl phosphate

6. acetyl phosphate + CoA → acetyl CoA

7. diacetyl $\xrightarrow{\text{NADH} \rightarrow \text{NAD}}$ acetoin

8. citrate → oxaloacetate + acetate

9. oxaloacetate → pyruvate + CO_2

10. threonine → glycine + acetaldehyde

11. acetaldehyde $\xrightarrow[\text{NADH} \rightarrow \text{NAD}]{}$ ethanol

12. acetaldehyde $\xrightarrow{\text{NAD} \rightarrow \text{NADH}}$ acetate

13. acetaldehyde $\xrightarrow{\text{NADH} \rightarrow \text{NAD}}$ ethanol

14. pyruvate + "active aldehyde" → acetolactate

15. acetolactate → acetoin + CO_2

16. acetoin $\xrightarrow{\text{NADH} \rightarrow \text{NAD}}$ 2,3,butylene glycol

17. threonine → glycine + acetaldehyde

18. pyruvate → acetaldehyde + CO_2

19. acetaldehyde $\xrightarrow{\text{NADH} \rightarrow \text{NAD}}$ ethanol

20. acetaldehyde + "active aldehyde" → acetoin

Threonine metabolism is responsible for the acetaldehyde accumulation. (Fig. 2, reaction 17). *Lb. bulgaricus* is species largely responsible for the formation of acetaldehyde in yogurt although both are able to convert threonine to glycine as they possess enzyme threonine aldolase.

Diacetyl and acetoin result from methabolic activity of *Str. thermophilus* and are very low, only 0.5 ppm. (Fig. 2, reaction 2,3,7). The presence of diacetyl contributes to the delicate, full flavour and aroma of yogurt and is especially important if acetaldehyde is low because it can enhance yogurt flavour. Pyruvate also may be metabolised via "active aldehyde" to acetoin (Fig. 2, reactions 14,15).

Many starter organisms metabolise acetaldehyde to ethanol (fig. 2, reaction 11). The lack of alcohol dehydrogenase, the enzyme catalysing this reaction, in both *Str. thermophilus* and *Lb. bulgaricus* makes these starters incapable of metabolizing acetaldehyde to ethanol, which means that acetaldehyde is the end metabolite.

Optimum flavour and aroma is obtained between 23 and 41 ppm acetaldehyde (Davies and Law, 1984).

The concentration of these typical flavour compounds changed during storage depending on duration and temperature in depot. The aim of this study was to show changes in content of flavour compounds and sensory evaluation occuring during storage as a function of duration and temperature. The aim was also to establish correlations between them.

Material and methods

In this article six commercial brands of plain yogurt were stored within a 10 days period at two different temperatures (+4°C; + 20° C) and every two days changes in acetaldehyde, diacetyl and ethanol were established. At the same time sensory evaluation was carried out.

Acetaldehyde and ethanol concentration was determined by aldehyde dehydrogenase and alcohol dehydrogenase method (Boehringer, 1989).

Diaceyl was measured by Hill's modification of the colorimetric method (Hill et al, 1954).

Five members' panel performed sensory analysis using scoring system based on weighted factors in the 20-points scale (ISO, 1985), and data analysed applying correlation and regression analysis (SAS/STAT, 1989).

Results and discussion

Investigation results are summarized in tables 1-4.

The results in table 1 show decrease in concentration of acetaldehyde during storage at both temperature levels in all yogurt samples. The storage temperature level significantly influence the decrease of acetaldehyde content.

The results in table 2 show the increase in concentration of diacetyl during storage at both temperature levels that do not influence the increase in concentration significantly.

Table 1 Concentration of acetaldehyde (ppm) in yogurt samples during storage at two temperature levels
Tablica 1. Udio acetaldehida (ppm) u uzorcima jogurta tijekom skladištenja pri dvije temperature

Samples Uzorci	T (°C)	Storage duration (days) Trajanje skladištenja (dani)				
		0	2	4	7	10
U-1	+4	7.50	7.04	6.48	5.23	2.84
	+20		6.36	6.13	5.00	1.36
U-2	+4	10.00	9.20	9.20	8.63	7.27
	+20		6.70	6.48	5.16	4.20
U-3	+4	10.45	8.18	6.25	5.45	5.27
	+20		7.11	5.00	4.43	3.07
U-4	+4	9.20	9.09	7.27	6.70	4.54
	+20		8.98	5.34	6.59	3.29
U-5	+4	5.34	4.32	3.86	3.63	3.18
	+20		4.09	3.52	2.95	2.84
U-6	+4	6.36	5.68	5.00	3.52	3.11
	+20		5.11	4.77	2.98	2.91

Table 2 Concentration of diacetyl (ppm) in yogurt samples during storage at two temperture levels
Tablica 2. Udio diacetila (ppm) u uzorcima jogurta tijekom skladištenja pri dvije temperature

Samples Uzorci	T (°C)	Storage duration (days) Trajanje skladištenja (dani)				
		0	2	4	7	10
U-1	+4	13.08	13.49	19.97	21.99	23.20
	+20		14.03	19.43	22.12	22.26
U-2	+4	14.06	14.30	17.67	17.94	23.47
	+20		14.70	16.46	19.29	22.26
U-3	+4	12.00	12.27	14.79	17.38	22.12
	+20		12.83	14.79	17.42	20.24
U-4	+4	10.65	16.01	16.61	20.65	20.73
	+20		17.42	17.68	19.17	23.07
U-5	+4	4.18	4.24	5.26	6.07	7.68
	+20		4.18	5.26	6.64	7.75
U-6	+4	6.83	11.06	13.22	13.62	14.97
	+20		13.08	13.22	13.62	14.84

Table 3 Concentration of ethanol (ppm) in yogurt samples during storage at two temperture levels
Tablica 3. Udio etanola (ppm) u uzorcima jogurta tijekom skladištenja pri dvije temperature

Samples Uzorci	T (°C)	Storage duration (days) Trajanje skladištenja (dani)				
		0	2	4	7	10
U-1	+4	1.38	1.99	2.42	3.01	3.34
	+20		2.38	3.22	3.87	4.26
U-2	+4	4.61	5.05	5.41	7.12	8.88
	+20		5.91	7.02	8.43	9.73
U-3	+4	2.42	2.67	2.99	5.27	7.55
	+20		2.78	3.06	6.00	8.75
U-4	+4	1.38	1.52	1.84	2.54	3.17
	+20		2.15	2.88	4.18	5.07
U-5	+4	1.50	2.22	2.84	3.92	4.84
	+20		2.39	3.11	4.15	5.18
U-6	+4	2.07	3.11	4.05	5.51	6.79
	+20		3.74	5.32	6.67	7.81

Table 4 Sensory evaluation (scores) of yogurt samples during storage at two temperture levels
Tablica 4. Senzorska procjena (bodovi) uzoraka jogurta tijekom skladištenja pri dvije temperature

Samples Uzorci	T (°C)	Storage duration (days) Trajanje skladištenja (dani)				
		0	2	4	7	10
U-1	+4	20.0	19.7	18.2	16.2	15.5
	+20		18.4	16.5	14.0	11.3
U-2	+4	20.0	19.9	19.4	18.9	18.6
	+20		18.9	17.6	14.6	12.9
U-3	+4	19.3	19.0	17.8	16.0	14.8
	+20		17.9	16.2	13.9	11.6
U-4	+4	18.5	17.8	17.4	16.9	16.0
	+20		16.6	14.5	13.8	12.7
U-5	+4	19.1	18.6	17.8	16.5	15.9
	+20		18.0	17.2	16.2	12.8
U-6	+4	19.7	19.3	18.9	18.2	17.1
	+20		18.2	16.9	16.1	14.7

Table 3. shows the increase in concentration of ethanol in yogurt samples during storage at both temperature levels which significantly influence ethanol increase.

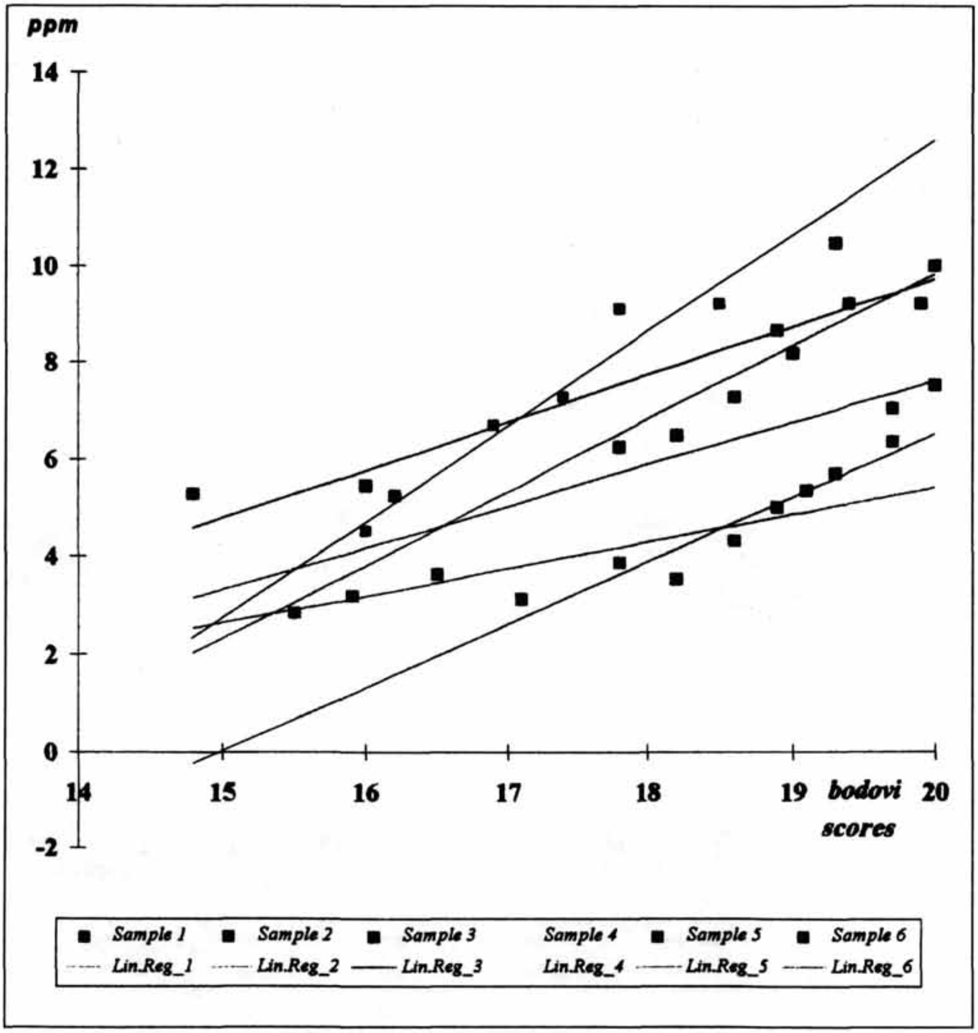

Figure 3 Correlation between sensory evaluation and acetaldehyde content in yogurt samples during 10 days storage at +4°C

Slika 3. Korelacija između senzorske ocjene i udjela acetaldehida u uzorcima jogurta tijekom desetodnevnog skladištenja pri +4°C

Sensory scoring (table 4) pointed out significant changes in yogurt quality during storage at both temperature levels those changes were pronounced on higher temperature levels.

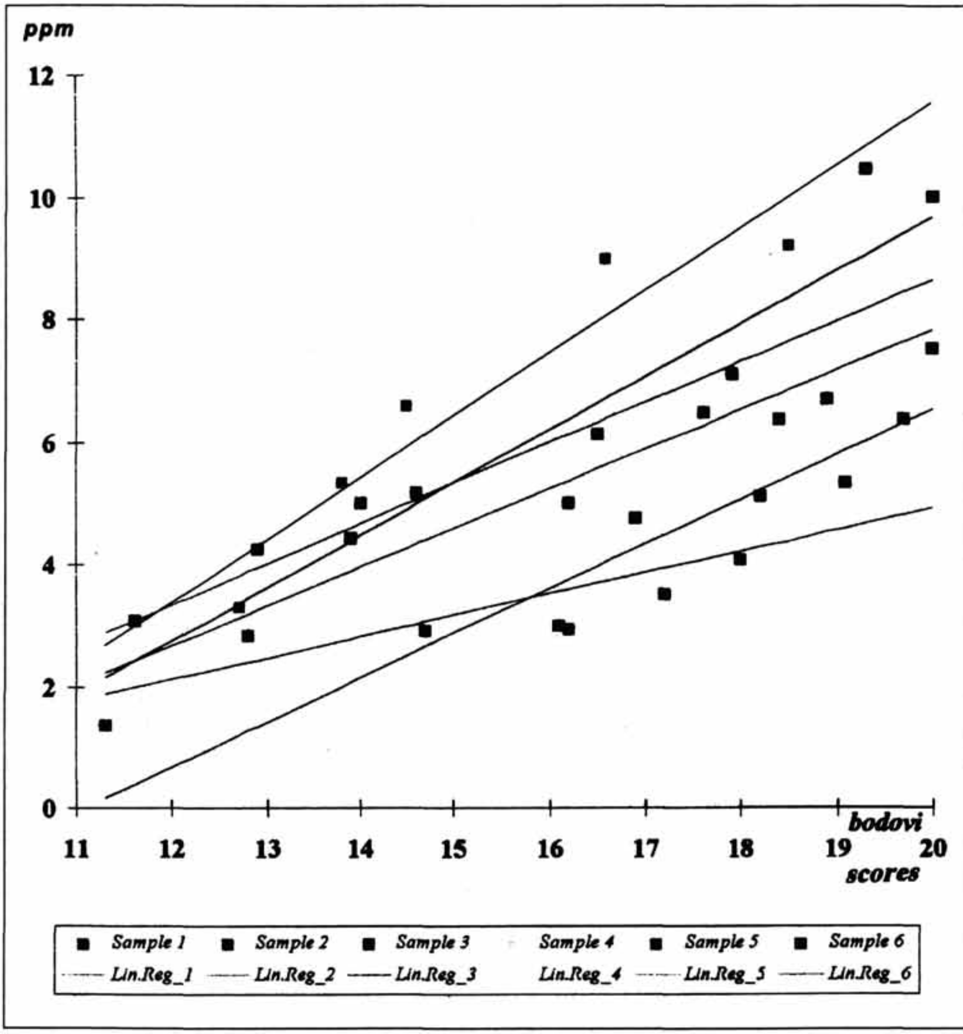

Figure 4 Correlation between sensory evaluation and acetaldehyde content in yogurt samples during 10 days storage at +20°C

Slika 4. Korelacija između senzorske ocjene i udjela acetaldehida u uzorcima jogurta tijekom desetodnevnog skladištenja pri +20°C

Sensory evaluations were regressed against the concentrations of aroma compounds at both temperature levels. Individual data points were plotted as well as the least squares fitted lines of all data (Figures 3-8).

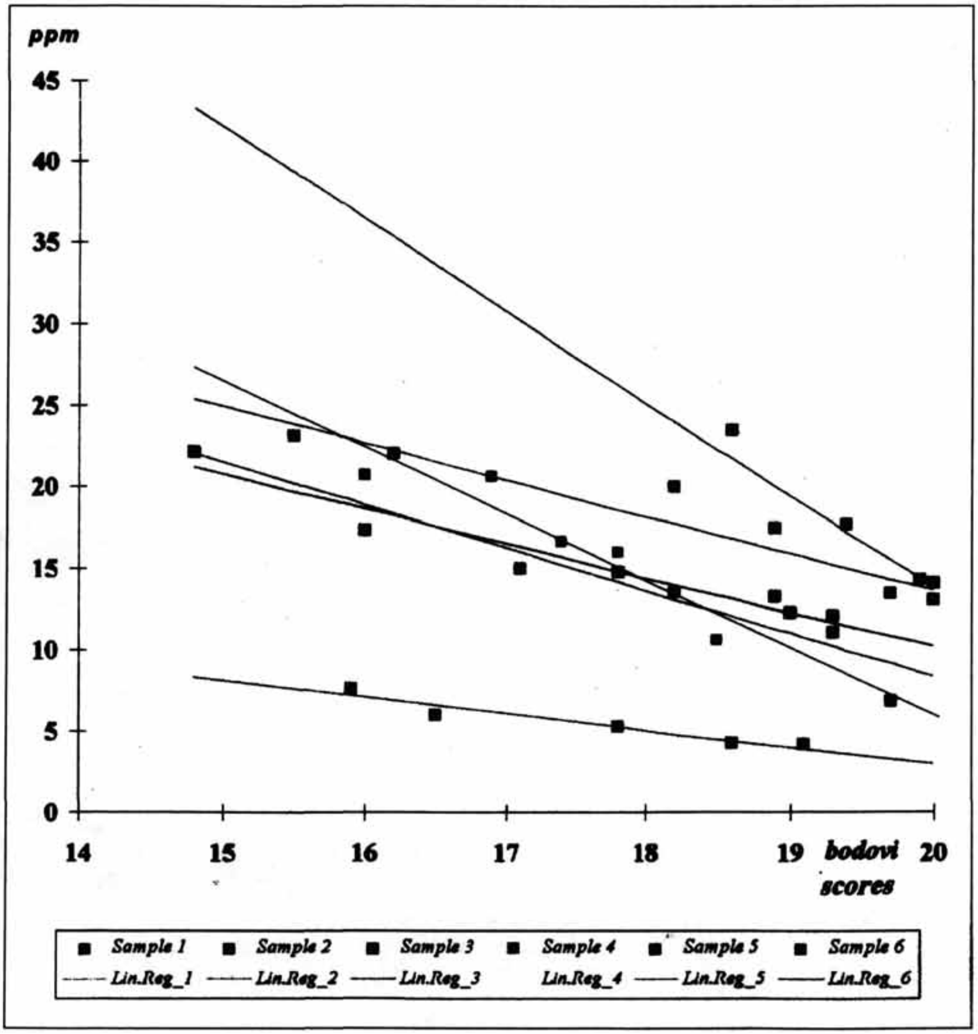

Figure 5 Correlation between sensory evaluation and diacetyl content in yogurt samples during 10 days storage at +4°C

Slika 5. Korelacija između senzorske procjene i udjela diacetila u uzorcima jogurta tijekom desetodnevnog skladištenja pri +4°C

Figure 3 and 4 show relationship between sensory evaluation and acetaldehyde content. Statistical analysis was performed and high values of correlation coefficients obtained. Regression lines were used as a measure of the linear relationship for both temperature levels.

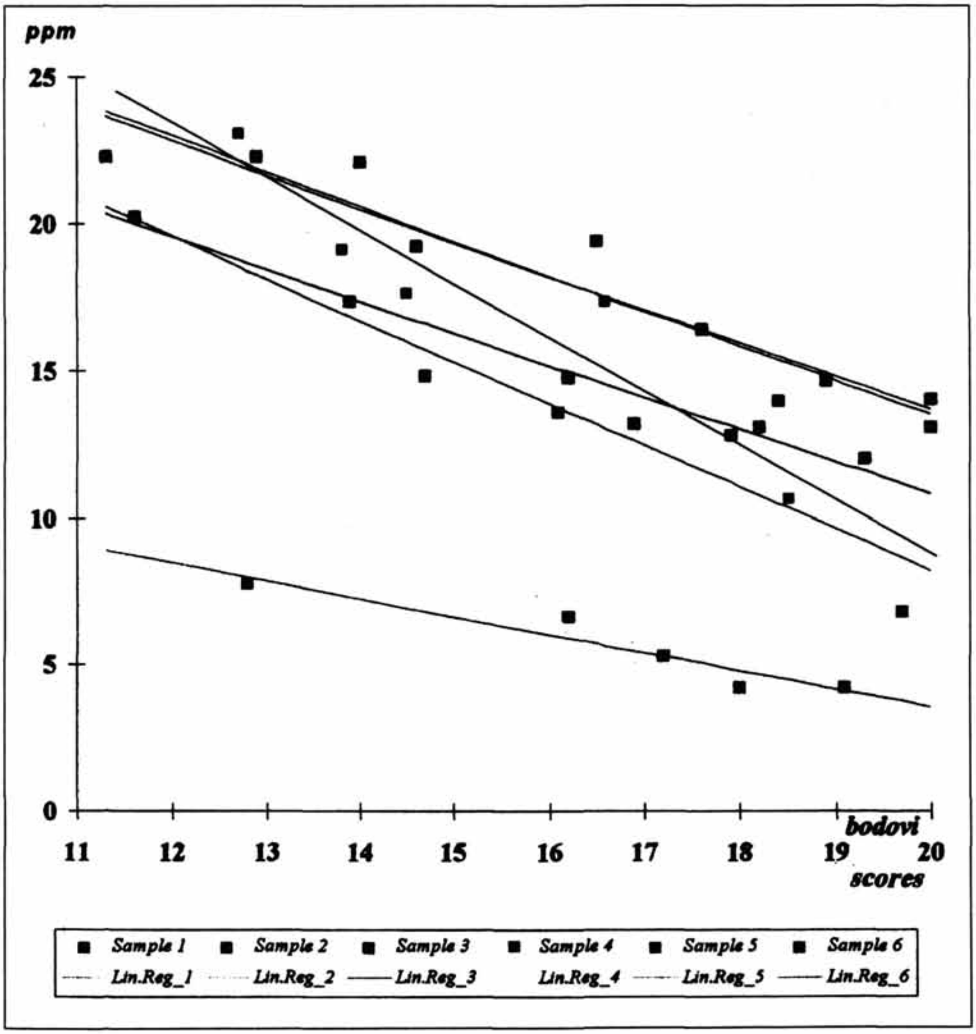

Figure 6 Correlation between sensory evaluation and diacetyl content in yogurt samples during 10 days storage at +20°C

Slika 6. Korelacija između senzorske ocjene i udjela diacetila u uzorcima jogurta tijekom desetodnevnog skladištenja pri +20°C

Sensory evaluation were correlated to diacetyl contents. High correlation coefficients values characterized linear relationship presented in figures 5 and 6 at both temperature levels.

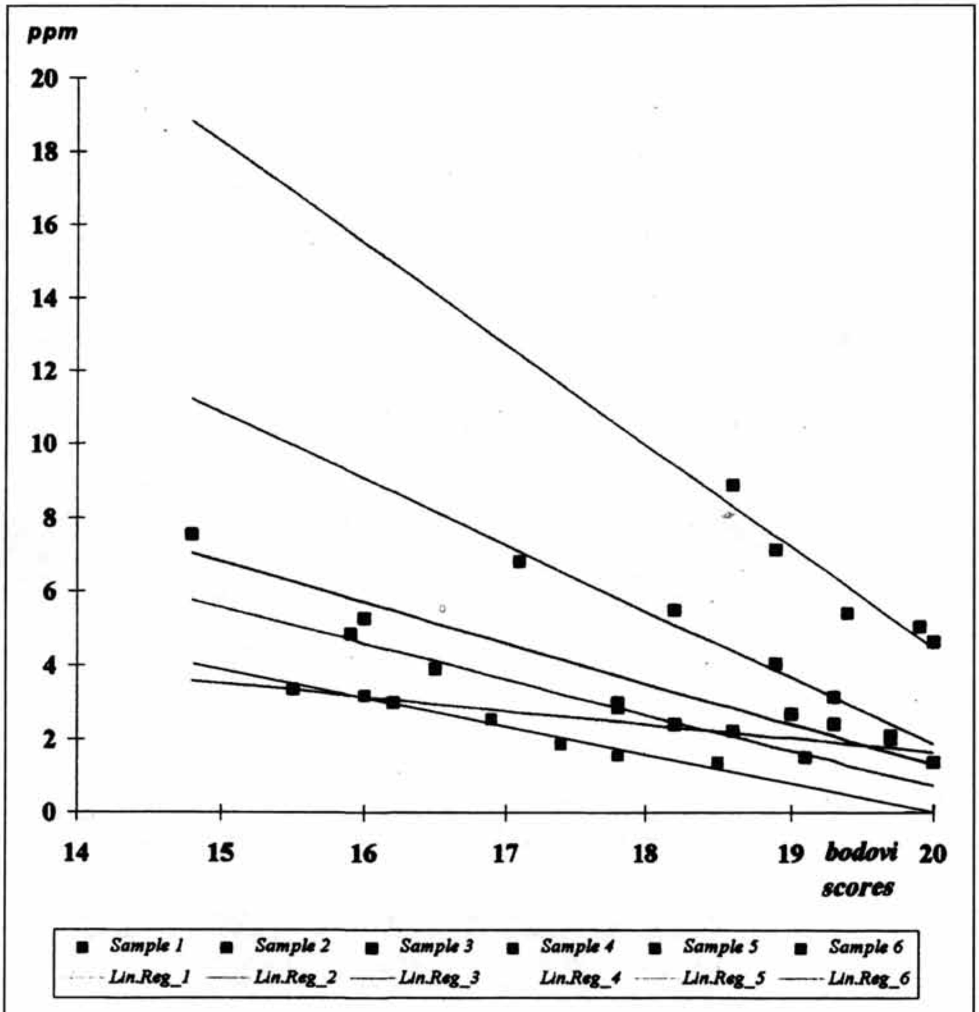

Figure 7 Correlation between sensory evaluation and ethanol content in yogurt samples during 10 days storage at +4°C

Slika 7. Korelacija između senzorske procjene i udjela etanola u uzorcima jogurta tijekom desetodnevnog skladištenja pri +4°C

The data of sensory evaluation and ethanol content were plotted as shown in figures 7 and 8 for all temperature levels, regression equations determined again showing very high linear correlation coefficients.

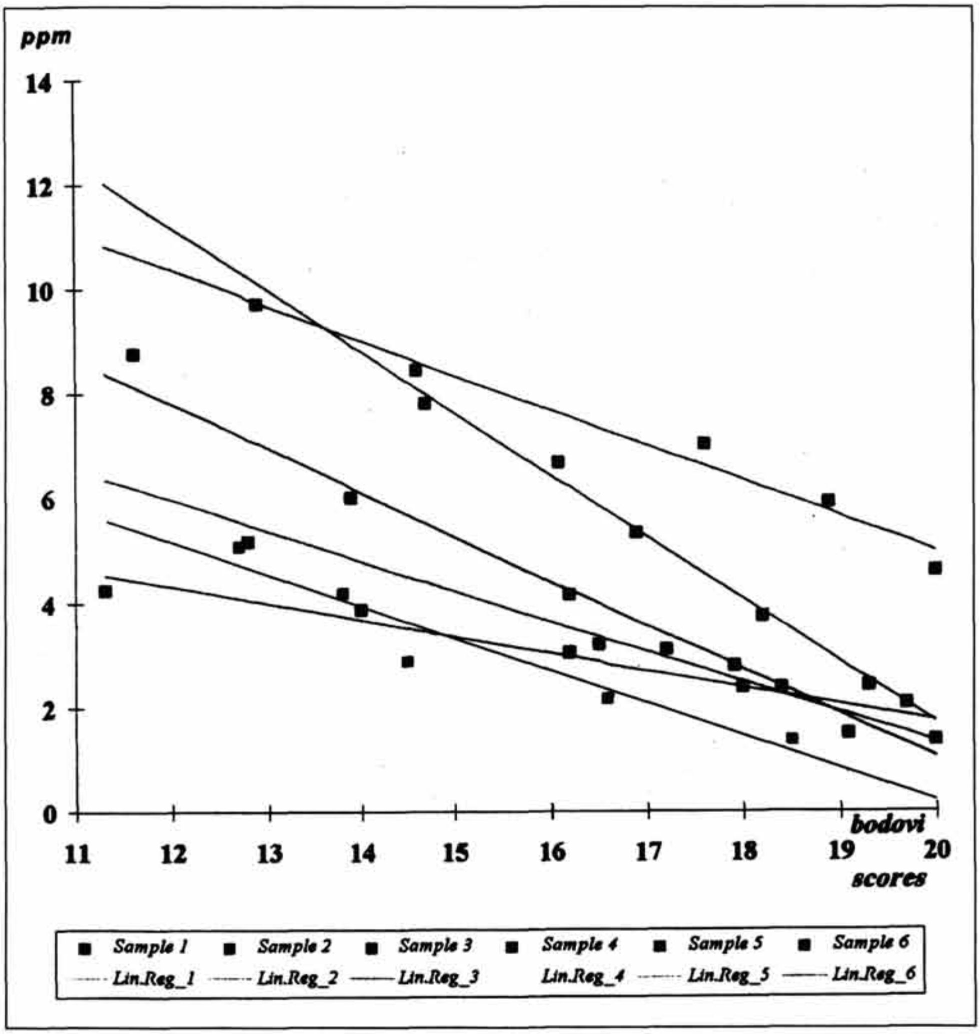

Figure 8 Correlation between sensory evaluation and ethanol content in yogurt samples during 10 days storage at +20°C

Slika 8. Korelacija između senzorske procjene i udjela etanola u uzorcima jogurta tijekom desetodnevnog skladištenja pri +20°C

Statistical parameters of regression analysis were summarized and presented in tables 5 and 6.

Sensoric evaluation changes are highly correlated to changes in acetaldehyde,diacetyl and ethanol contents respectively. The best correlation was between sensory evaluation and ethanol content at both temperature levels (from -0.956 to 0.996), followed by correlation coefficients for sensory evaluation and diacetyl content also at both temperature levels (from-0.849 to - 9.996). Other coefficients of correlation were high as well. The recorded values for this statistic parameter were generally between 0.824 and 0.996.

Table 5 Correlation coefficients and coefficients of determination between sensory evaluation and concentration of acetaldehyde, diacetyl and ethanol, respectively
Tablica 5. Koeficijenti korelacije i koeficijenti determinacije između senzorske procjene i udjela acetaldehida, diacetila i etanola

	samples uzorci	T (°C)	Aroma compounds (ppm) Komponente arome (ppm)					
			acetaldehyde acetaldehid		diacetyl diacetil		ethanol etanol	
			r	R^2	r	R^2	r	R^2
SENSORY EVALUATION	U-1	+4	0.930	0.865	-0.963	0.928	-0.972	0.944
		+20	0.944	0.891	-0.934	0.871	-0.963	0.928
	U-2	+4	0.905	0.819	-0.932	0.868	-0.956	0.914
		+20	0.897	0.804	-0.992	0.984	-0.988	0.976
	U-3	+4	0.872	0.761	-0.981	0.963	-0.969	0.939
		+20	0.924	0.854	-0.996	0.992	-0.958	0.917
	U-4	+4	0.996	0.993	-0.926	0.858	-0.970	0.940
		+20	0.947	0.897	-0.938	0.880	-0.957	0.916
	U-5	+4	0.919	0.845	-0.964	0.929	-0.994	0.988
		+20	0.824	0.679	-0.949	0.900	-0.961	0.923
	U-6	+4	0.958	0.918	-0.849	0.721	-0.986	0.971
		+20	0.952	0.906	-0.867	0.752	-0.996	0.992

Table 6 Regression equations for relationship between sensory evaluation and concentration of acetaldehyde, diacetyl and ethanol, respectively
Tablica 6. Jednadžbe regresije za povezanost između senzorske ocjene i udjela acetaldehida, diacetila i etanola

	samples uzorci	T (°C)	Aroma compounds (ppm) Komponente arome (ppm) acetaldehyde acetaldehid	diacetyl diacetil	ethanol etanol
SENSORY EVALUATION / SENZORSKA OCJENA	U-1	+4	Y=-9.57+0.86x	Y=58.98-2.27	Y=9.17-0.38x
		+20	Y=-5.03+0.64x	Y=37.13-1.18x	Y=8.20-0.32x
	U-2	+4	Y=-20.22+1.50x	Y=129.89-5.81x	Y=59.81-2.77x
		+20	Y=-4.57+0.66x	Y=36.47-1.14x	Y=18.41-0.67x
	U-3	+4	Y=-10.00+0.98x	Y=52.58-2.12x	Y=23.26-1.10x
		+20	Y=-7.58+0.86x	Y=32.74-1.09x	Y=17.93-0.85x
	U-4	+4	Y=-26.78+1.97x	Y=87.50-4.07x	Y=15.49-0.77x
		+20	Y=-8.82+1.02x	Y=45.23-1.82x	Y=12.52-0.62x
	U-5	+4	Y=-5.71+0.56x	Y=23.62-1.03x	Y=20.19-0.97x
		+20	Y=-2.08+0.35x	Y=15.91-0.62x	Y=12.88-0.58x
	U-6	+4	Y=-19.51+1.30x	Y=61.16-2.64x	Y=38.02-1.81x
		+20	Y=-8.09+0.73x	Y=36.62-1.42x	Y=25.40-1.18x

Conclusion

Concentration of acetaldehyde decreased, while concentrations of diacetyl and ethanol increased in all yogurt samples during storage at both temperature levels.

Temperature level in depot significantly influenced the decrease of acetaldehyde content and the increase of concentration of ethanol but not significantly the increase of diacetyl content during storage.

Sensory scoring pointed out significant changes in quality of all yogurt samples at both temperature levels. Changes were pronounced during storage at higher temperature level.

Regression analysis determined linear relationship between sensory evaluation and concentrations of all investigated aroma compounds.

Coefficients of correlation were generally high and consequently very accurately predicting equations obtained by regression analysis.

SASTOJCI AROME I SENZORSKA OCJENA JOGURTA TIJEKOM SKLADIŠTENJA

Sažetak

Poznato je da od stotinjak kemijskih spojeva izoliranih iz jogurta i sličnih fermentiranih proizvoda samo neki od njih (acetaldehid, etanol, diacetil i 2-butanon) znatno utječu na željeni okus proizvoda. Koncentracija tih tipičnih sastojaka arome mijenja se tijekom skladištenja ovisno o temperaturi i trajanju.

U ovom radu šest uzoraka jogurta čuvano je pri dvije različite temperature tijekom 10 dana i svaka dva dana uzimani su uzorci za određivanje acetaldehida, diacetila i etanola. Istovremeno je jogurt senzorski ocjenjen. Udio acetaldehida i etanola određen je enzimnom metodom a udio diacetila modificiranom Hill-ovom kolorimetrijskom metodom. Senzorski je jogurt ocijenila skupina od 5 članova koristeći sistem 20 ponderiranih bodova. Utvrđena je kvantitativna ovisnost senzorske ocjene i svake pojedine komponente arome za obje razine temperature i izražena linearnim jednadžbama. Najbolja povezanost, izražena koeficijentom korelacije, utvrđena je između senzorske procjene i etanola (r = 0.996) u jednom od uzoraka čuvanom pri +4°C. Općenito svi su koeficijenti korelacije bili visoki (od 0,824 do -0,996).

Ključne riječi: komponente arome, acetaldehid, diacetil, etanol, senzorska procjena, korelacija.

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