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Chemical Nature of Malty Flavor and Aroma Produced by *Streptococcus lactis* var. *maltigenes*[1]

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Mature skim milk cultures of *Streptococcus lactis* var. *maltigenes* were steam distilled at low temperature under reduced pressure. Ethyl ether extracts were prepared from the distillates and analyzed by gas-liquid chromatography and mass spectrometry. Twenty of 31 components detected in the culture distillates were identified positively and 11 tentatively, whereas 10 of 19 components detected in the heated skim milk control were identified positively and 9 tentatively. Among components detected in the culture distillate, but not detected in the heated skim milk distillate, and which have not been previously identified in milk cultures of the organism were phenylacetaldehyde and phenethanol. Quantitative analyses of the volatiles entrained from milk cultures of several strains of *S. lactis* var. *maltigenes* revealed a probable relationship between variation in the character of the aroma of the cultures and the alcohol/aldehyde ratio.

The conversion of leucine to 3-methylbutanal by *Streptococcus lactis* var. *maltigenes* was first implicated as being primarily responsible for the malty flavor and aroma produced in milk cultures (2). Later work revealed that the organism possesses transaminase and decarboxylase systems which mediate the conversion of several amino acids to the corresponding aldehydes (4, 5, 10). These observations along with more recent analyses of culture volatiles (6) suggest that the malty character may be an expression of several components in addition to that of 3-methylbutanal. The present investigation provides further information concerning the flavor and aroma produced in milk by *S. lactis* var. *maltigenes*.

MATERIALS AND METHODS

Cultures of strains of *S. lactis* var. *maltigenes* from the collection described by Gordon et al. (1) designated M1, RM2, M3, and P25 were maintained in sterile reconstituted antiobiotic-free nonfat dry milk medium. Transfers were made every third day by using 1% inoculum. Cultures were incubated at 30 C until coagulation occurred (18 to 20 hr) and were then stored at 2 C. Culture for distillation was prepared in 37.8-liter lots of fresh skim milk (8.5% solids-nonfat) heated at 96 C for 1 hr in stainless-steel cans. The milk was cooled to 30 C, inoculated with 1% active culture, and incubated for 18 hr.

Seventy-five-liter quantities of culture M1 and a heated skim milk control (acidified to *p*H 5.2 with 10% phosphoric acid) were steam distilled at a pressure of 2 mm of Hg (3). Approximately 10% of the culture volume was recovered as distillate. This was extracted with peroxide-free ethyl ether (11) for 24 hr in a conventional liquid-liquid extractor (8.5 by 60 cm extraction chamber). The extracts were dried over anhydrous sodium sulfate and fractionally distilled to remove the excess ether.

Components of the extracts were separated by gas-liquid chromatography (GLC) on two columns of differing polarity and tentatively identified by coincidence of relative retention times with those of known compounds. The columns were 305 cm by 3.175 mm outer diameter stainless-steel packed with 20% diethyleneglycol succinate on 80 to 100 mesh, acid-alkali-treated Celite 545, and uncoated, 100 to 120 mesh Poropak Q and were temperature programmed as follows: 60 C for 15 min, then 4 C/min to 200 C; 4 C/min from 100 to 210 C, respectively. Nitrogen was the carrier gas (25 cc/min and 20 cc/min measured at the starting temperature), and the separated components were detected by flame ionization.

To achieve greater resolution of components prior to mass spectral analysis, the extracts were chromatographed on 91.44 m by 0.254 mm open tubular columns coated with either Apiezon L or butanediol succinate and temperature programmed as follows: 60 C for 15 min, and then 4 C/min to 200 C; 125 C for 10 min, and then 4 C/min to 200 C; respectively.

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TABLE 1. *Flavor compounds identified in the malty culture and heated skim milk by gas chromatography (GLC) and mass spectrometry (MS)*

Compound	Identified in			
	Malty culture		Heated skim milk	
	GLC	MS	GLC	MS
Aldehydes				
Acetaldehyde	+	+	+	+
2-Methylpropanal	+			
Pentanal	+		+	
3-Methylbutanal	+	+		
Hexanal			+	
Octanal			+	
Benzaldehyde	+		+	+
Nonanal			+	
2-Furfural	+		+	+
Phenylacetaldehyde	+	+		
Alcohols				
Ethanol	+	+		
Butanol		+		
2-Methylpropanol	+	+		
3-Methylbutanol	+	+		
2-Furfurol	+	+	+	+
Phenethyl alcohol	+	+		
Methyl ketones				
Acetone	+	+		
Butanone	+	+		
2-Pentanone	+	+	+	+
2-Hexanone			+	
2-Heptanone	+	+	+	+
2-Octanone			+	
2-Nonanone	+	+	+	+
2-Undecanone	+		+	+
2-Tridecanone	+		+	+
Esters				
Ethyl formate	+	+	+	
Ethyl acetate	+	+	+	+
Ethyl butyrate	+	+		
Ethyl isovalerate		+		
Ethyl hexanoate	+			
Ethyl octanoate	+	+	+	
Ethyl decanoate	+			
Methyl acetate	+		+	
Isoamyl acetate		+		
Acids				
Formic	+			
Acetic	+			

Helium, at a flow rate of about 1 cc/min, served as the carrier gas. The column effluents were directed into the ionization chamber of an Atlas CH4 mass spectrometer operated at 1.5×10^{-6} mm of Hg. A 20-ev source was employed as a chromatographic detector and the 70-ev source provided the mass spectra. Spectra were recorded from mass 25 to 250 in 2 or 4.5 sec with a Honeywell Visicorder (model 1508). The identification of components was considered positive when confirmed by mass spectral analysis.

TABLE 2. *Evaluation of the aroma over 24-hr-old skim milk cultures of four strains of Streptococcus lactis var. maltigenes*

Strain	Evaluation
M1	Balanced malty aroma; smooth
RM2	Aroma is not balanced but of equal malty intensity as compared with M1; harsh.
M3	Slightly malty
P25	Aroma of a typical *S. lactis* culture; little, if any, malty character.

Quantitative analyses of the volatiles produced in skim milk by different strains of *S. lactis* var. *maltigenes* were made by an on-column trapping GLC technique (7) as employed by Morgan et al. (8). A Varian-Aerograph (model 1200) gas chromatograph equipped with a hydrogen flame detector and a column (366 cm by 3.175 mm outer diameter) packed with 20% 1,2,3-tris-(2-cyanoethoxy)propane on 80 to 100 mesh, acid-alkali-washed Celite 545 was used for the analyses. The column was operated isothermally at 55 C and adequately separated the volatile constituents. To obtain quantitative data, the GLC recorder response for each sample component was compared to that obtained for graduated concentrations of the pure compound in skim milk.

A subjective evaluation of the aroma and flavor produced in skim milk culture by strains of *S. lactis* var. *maltigenes* and by addition of mixtures of authentic compounds was made by a laboratory panel of three members who were familiar with the malty aroma. A 10-member trained panel was employed in determination of the average flavor threshold (9) in both skim milk and water of pure compounds found to be associated with the malty cultures.

RESULTS AND DISCUSSION

In the summary of the volatiles identified in the extracts of the distillates from the malty culture and the heated skim milk (Table 1), it will be noted that certain aldehydes, alcohols, and esters were detected only in the extract obtained from the culture distillate. Along with 2-methylpropanal, 2-methylpropanol, 3-methylbutanal, and 3-methylbutanol, all of which have been previously detected in milk cultures of this organism (8), phenylacetaldehyde, phenethanol, and several simple esters were detected. Since the conversion of several amino acids including phenylalanine to the corresponding aldehydes by resting cells of the organism has been demonstrated (6) and since a phenylacetaldehyde-like note is occasionally detected in the aroma of malty cultures, the detection of phenylacetaldehyde in a vacuum distillate of the culture employed in this study was not unexpected. The alcohols present in the culture distillate undoubtedly resulted from a reduction

TABLE 3. *Quantitative analysis of volatiles entrained from 24-hr-old skim milk cultures of Streptococcus lactis var. maltigenes with alcohol/aldehyde ratios and developed acidity*

Volatile compound	Concn in strain			
	RM3	M	M3	P25
Ethanol / Acetaldehyde	31.03[a] / 3.30 = 9.0	56.34 / 0.17 = 331.4	195.62 / 1.74 = 112.4	22.45 / 6.00 = 3.7
2-Methylpropanol / 2-Methylpropanal	2.07 / 1.87 = 1.1	1.79 / 0.57 = 3.1	2.98 / 0.50 = 6.0	0.20 / 0.50 = 0.4
3-Methylbutanol / 3-Methylbutanal	7.18 / 4.45 = 1.6	9.01 / 2.85 = 3.2	14.58 / <3.00 = >4.9	1.37 / <1.00 = >1.4
Acetone	0.28	0.28	0.32	0.32
Titratable acidity	1.04[b]	1.07	0.78	0.75

[a] Concentrations expressed as micrograms per milliliter.
[b] Expressed as per cent lactic acid.

TABLE 4. *Average flavor thresholds for some volatile compounds present in malty cultures*

Compound	Avg flavor thresholds[a]	
	Water	Skim milk
2-Methylpropanal	0.18	0.10
2-Methylpropanol	5.25	5.00
2-Methylbutanal	0.04	0.13
2-Methylbutanol	5.50	6.25
3-Methylbutanal	0.06	0.06
3-Methylbutanol	4.75	3.20
Phenylacetaldehyde	0.04	0.02
Phenethyl alcohol	0.24	0.07

[a] Expressed as micrograms per milliliter.

of the corresponding aldehydes mediated by the alcohol dehydrogenase known to be active in this organism (G. A. Harrison, E. H. Khairallah, and M. E. Morgan, Bacteriol. Proc., p. 134, 1969).

Although appreciable ethanol, other alcohols, and probably some volatile acids were produced in the culture, it is doubtful that esters detected in the extract of the distillate were produced by the organism. None of these esters has been detected in analyses of malty cultures by the entrainment on-column trapping GLC technique (9) and ester-like notes are not detectable in the aroma or flavor of mature malty cultures. It appears, therefore, that the esters detected in the present study were formed during the extraction of the culture distillate or concentration of the extract.

The subjective evaluation of the aroma over 24-hr-old skim milk cultures of four strains of *S. lactis* var. *maltigenes* is shown in Table 2. Although each of these strains produced titratable acidities in excess of 0.6% expressed as lactic acid and possess an active α-keto acid decarboxylase system (Table 3), they represent extremes in respect to these criteria (1), i.e., strains M1 and RM2 produced a more pronounced malty aroma and attained an appreciably higher titratable acidity than did M3 and P25.

The quantitative analyses of the volatile compounds entrained from the mature skim milk cultures as determined by GLC are shown in Table 3. Variation in the quantities detected are apparent and appear to be related to the flavor and aroma of the cultures. Since the average flavor threshold of the aldehydes is much lower than that of the corresponding alcohols (Table 4), it appears that the decreasing intensity of the aroma observed in cultures RM2, M1, and M3, respectively, is due to increases in the alcohol/aldehyde ratios (ethanol/acetaldehyde, 2-methylpropanol/2-methylpropanal, 3-methylbutanol/3-methylbutanal). This loss of intensity in flavor and aroma is undoubtedly related to an aldehyde to alcohol conversion as exemplified by the relatively weak aroma of strain M3. Strain M1 had a moderately intense but smooth aroma which would be indicative of a balance between the alcohols and aldehydes. The aroma of strain RM2 possessed a harshness which suggested a high aldehyde content or a low alcohol/aldehyde ratio. Although strain P25 produced a relatively large amount of acetaldehyde, it produced only traces of 2-methylpropanal and 3-methylbutanal. Despite the low conversion of aldehydes to alcohols, the aroma of the culture possessed but little malty character. Qualitatively and quantitatively, the volatiles entrained from this culture resemble those from a number of other weakly malty or

nonmalty cultures of *S. lactis* examined previously (8).

Threshold values of the authentic compounds (Table 4) indicate that all of the compounds (except ethanol and acetone) detected by GLC in the volatiles entrained from the cultures are probably essential to the characteristic aroma developed in mature cultures. This does not contradict the earlier hypothesis that 3-methylbutanal is the primary odor constituent in immature cultures of *S. lactis* var. *maltigenes* (2) or the early stages of the malty defect as might be detected in producer samples of raw milk (6). The parameters employed in the GLC analyses of the volatiles entrained from the culture did not permit detection of higher boiling compounds such as phenylacetaldehyde and phenethanol. These compounds undoubtedly contribute to the flavor and aroma of cultures of the organism, especially in view of their low flavor threshold in water and skim milk (Table 4).

To evaluate further the flavor character of the compounds in the GLC analyses of entrained culture volatiles, a synthetic flavor formulation was prepared by using the quantitative data for the M1 culture. Milk containing the mixture of flavor compounds possessed a distinct malty flavor and aroma although it lacked the overall character of mature M1 culture. The flavor character could be easily manipulated by varying concentrations of aldehydes and alcohols and by adding phenylacetaldehyde and phenethanol. The aroma variations were quite similar to those observed during different stages of growth of the organism. Although the flavor and aroma of *S. lactis* var. *maltigenes* cultures is usually described as malty, other terms such as burnt, caramel, and "grapenuts" have been employed. In view of the potentially desirable flavors from these cultures, and their similarity to volatile yeast metabolites, it is possible that this organism could be utilized in the production of natural flavors for food products.

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