out on a Varian aerograph Series 1 800, with 5 m × 0.125 in. stainless steel column, packed with 5% FFAP on Varaport 80–100 mesh. The column, was programmed from 70 °C at 2 °C/min to 250 °C. For sensory tests of individual peaks, we used an equal size column, packed with 12% FFAP. The column outlet was split into two fractions: about 7% of the gas flow passed the flame ionization detector and the rest went to the sniffing tube. The temperature was programmed from 90 °C at 2 °C/min to 250 °C. Mass spectrometric analysis were performed after fractionation of the flavor concentrates on silica gel by means of column chromatography as described previously (Schreier and Drawert, 1974).

RESULTS AND DISCUSSION

In the silica gel fractionated flavor extracts of *Kluyveromyces lactis* culture broths about 150 different compounds were detected by gas chromatography. Among the hitherto identified compounds are the monoterpenes citronellol, linalool, and geraniol which were identified with the aid of reference compounds. Samples, reference compounds, and samples with added references had identical properties in gas chromatography, in silica gel fractionation, in their sensory qualities (sniffing of single gas chromatographic peaks at the split outlet of the column), and in mass spectrometry. Citronellol and linalool accumulated at about 50 μg/L in the culture broth; geraniol, which is the first monoterpene formed via the mevalonic acid pathway (Banthorpe et al., 1972; Lanza and Palmer, 1977) could be detected only in traces. In experiments with added geraniol (0.05, 0.5, 5, and 15 mg/L) we found that geraniol is reduced nearly quantitatively (about 90%) to citronellol. Only 1–2% of the added geraniol remained unchanged after a culture period of 4 days.

Monoterpene biosynthesis by *Kluyveromyces lactis* does not depend on special precursors, but variations of culture conditions had an influence on their yield as we could show for citronellol. With increasing culture temperature (10, 15, 22, and 27 °C) citronellol was formed at a higher rate in a medium containing 20 g of glucose and 0.5 g of asparagine/liter, but no vitamins. These cultures were inoculated with washed yeast cells, grown for 30 h on an equal medium with vitamin supply. Relative yields under these conditions were 18, 22, 64, and 100%, respectively. The 100% value corresponds to 60 μg of citronellol/liter.

A similar effect was observed for increasing concentrations of asparagine (0.1, 0.2, 0.5, and 1 g/L) at a constant amount of 20 g of glucose/liter of culture medium. Citronellol yield rose with increasing asparagine concentration from 2% over 12% and 71% up to the final value 100%, respectively, 50 μg of citronellol/liter.

These results confirm previous findings that microorganisms are able to produce monoterpenes. By changing the culture conditions, it is possible to influence the biosynthesis of citronellol in the yeast *Kluyveromyces lactis*.

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Effect of Heat Treatment on Amino Acid Composition of Canned Baby Food

The amino acid content of some canned baby foods was analyzed before and after normal processing including heat treatment at 70 °C for 1 h and at 121 °C for 50 or 60 min. In all heated products there was a decrease in total amino acids in proteins, but the percentage proportion of essential amino acids in the total amino acids remained unchanged. Available lysine content decreased by approximately 15% in all foods studied. The decrease was also found in chemical score values, which were about 10% lower after heat treatment. Only traces of lysinoalanine were found in some samples.

It is a well-known fact that when protein foods or isolated proteins are heated above 100 °C at about neutral pH, chemical changes take place and reduce the nutritive value of the proteins (Bjarnason and Carpenter, 1970; Osner and Johnson, 1974, 1975; Hurrell and Carpenter, 1976). Similar changes have also been found in canned baby foods during sterilization (Rakowska, 1972). One of the products of damaged amino acids in proteins is lysinoalanine, especially after heat treatment in alkaline conditions (De Groot and Slump, 1969; Woodard and

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Table I

Baby food	Content
1. Strained fish and vegetables	Potato, carrot, saithe, corn starch, rice, and salt
2. Chopped fish and potato	Saithe, potato, skimmed milk powder, wheat flour, salt, and dill
3. Chopped chicken and vegetables	Chicken meat, carrot, peas, corn, potato, corn starch, paprika, tomato puree, lemon juice, and salt
4. Strained egg yolk liver, and potato	Potato, carrot, liver (beef and chicken), egg yolk, skimmed milk powder, and salt
5. Chopped beef and vegetables	Beef, potato, carrot, peas, tomato puree, corn starch, and salt

Short, 1973; Asquith and Carthew, 1972; Mauron, 1975). Lysinoalanine formation in nonalkaline conditions have been reported by Sternberg et al. (1975a).

This communication reports the characterization of the amino acid pattern of some canned baby foods of different composition before and after heat treatment in nonalkaline conditions.

MATERIALS AND METHODS.

The experimental materials, canned baby foods, were produced and marketed in Finland and their contents were as shown in Table I. Samples were taken during processing immediately after mixing. The processing procedure takes about 1 h and is performed at 70 °C, followed by sterilization at 121 °C for 50–60 min.

For the amino acid assays 1–2 kg of food mixture or canned product was homogenized thoroughly and a part of it was lyophilized. Two hundred milligrams of the lyophilized material was hydrolyzed in tightly closed glass bottles with 200 mL of 6 N HCl at 110 °C for 24 h. Oxygen was removed by passing nitrogen through the solution. The hydrolysate was evaporated to dryness in a rotary evaporator.

Amino acids were determined with a Hitachi amino acid analyzer model KLA-5. Norleucin served as an internal standard. Tryptophan was determined by a colorimetric method of Graham et al. (1947) and cystine and cysteine together as cysteic acid by a method of Schram et al. (1954). Available lysine was assayed by a method of Carpenter (1960) based on a dinitrophenyl derivative of lysine. Lysinoalanine assay was carried out as described by Sternberg et al. (1975b). Nitrogen content was determined by the Kjeldahl procedure from the lyophilized material. Chemical score values were computed from the limiting amino acids. Whole egg protein was used as a reference protein, which was analyzed in connection with the samples.

RESULTS AND DISCUSSION

The amino acid contents of five canned baby foods before and after heat treatments are given in Table II. A loss of total amino acids was observed in all heated products, varying between 9 and 20%. A similar loss was found in the essential amino acids. Usually no single amino acid was responsible to the loss observed. The amount of available lysine was reduced by 9 to 28%. The loss was highest in products containing vegetables and fish (no.'s 1 and 2). The decrease in chemical score values was of the order of 16–30% when the calculation was based on the limiting amino acids, which were valine or methionine and cysteine. Valine and methionine were determined in the acid hydrolyzate with amino acid analyzer. Cysteine was measured as cysteic acid as presented in the Materials and Methods section. Our results are in agreement with the

Table II. Amino Acid Content of Some Canned Baby Foods before and after Heat Treatments[a]

Amino acid, g/16 g of N	Baby food										Standard deviation%	Recov., %
	No. 1		No. 2		No. 3		No. 4		No. 5			
	A	B	A	B	A	B	A	B	A	B		
Alanine	4.4	2.8	3.3	3.1	4.2	4.3	3.6	2.7	4.6	4.6	9	72
Arginine	4.2	3.8	3.5	2.7	5.1	4.3	5.2	4.4	7.1	5.5	14	79
Aspartic acid	9.3	9.4	7.2	6.7	8.7	6.0	11.5	10.8	7.6	8.0	5	95
Cysteic acid	0.8	0.7	0.8	0.4	0.9	0.7	0.8	0.7	0.6	0.5	8	96
Glutamic acid	11.9	10.6	11.7	10.5	10.1	8.7	11.5	10.0	12.9	10.9	8	89
Glycine	3.5	2.8	3.3	2.7	4.5	4.4	2.6	2.0	3.4	2.9	5	89
Histidine	2.3	1.7	2.4	1.8	3.4	2.2	2.7	2.4	4.2	3.8	10	85
Isoleucine	3.5	2.7	4.4	4.0	4.4	3.7	3.9	4.5	3.0	3.4	4	90
Leucine	6.1	5.1	7.0	6.9	6.3	5.8	6.4	7.5	6.0	6.3	5	90
Lysine	4.8	5.3	5.6	4.4	4.6	4.4	5.0	4.2	9.0	6.0	8	74
Methionine	2.4	1.9	2.8	2.6	2.5	2.0	2.3	1.6	2.6	1.7	8	59
Phenylalanine	2.9	3.3	4.5	3.2	4.0	2.7	3.3	3.2	4.0	4.3	10	116
Proline	2.2		2.7	2.8	1.7		2.9	1.3	2.5	2.0		
Serine	5.9	4.7	3.5	3.4	3.2	3.2	3.9	3.1	3.3	2.9	6	77
Threonine	3.9	3.2	4.1	3.9	3.7	3.7	3.9	3.1	4.9	3.8	7	84
Tryptophan	1.1	1.1	1.1	0.9	1.0	1.2	1.0	1.0	0.8	0.8	8	107
Tyrosine	2.7	2.8	4.6	3.0	3.5	2.6	3.9	4.6	3.7	3.7	7	102
Valine	3.4	3.6	5.7	4.9	4.6	3.9	4.4	4.0	3.7	3.5	6	98
Av. Lys	5.1	3.6	5.0	3.9	4.8	3.9	4.6	4.3	6.8	5.8	2	85
Ess. aa	31.6	29.8	40.6	34.6	35.5	30.7	34.9	34.4	38.3	34.5		
Ess. %	42	44	52	50	47	51	45	48	46	46		
Tot. aa	75.6	66.7	77.4	67.9	75.1	59.7	77.9	71.1	83.9	75.1		
CS, Val	55	58										
CS, Met + Cys	57	48	65	54	61	48	56	41	57	40		

[a] A = before heat treatments, B = after heat treatments, Av. Lys = available lysine, Ess. aa = essential amino acids, Ess. % = percentage value of the essential amino acids compared to the total amino acid content, Tot. aa = total amino acids, CS, val = chemical score based on valine as limiting amino acid, CS, met + Cys = chemical score based on methionine and cysteine as limiting amino acids. For contents of canned baby foods no.'s 1–5, see the Materials and Methods section. All values are mean values of double or triple assays. Standard deviation ($n = 6$) and recovery (2.5 μmol/each amino acid was added) values were estimated for the baby food no. 2. Recovery % is a mean value of three estimates.

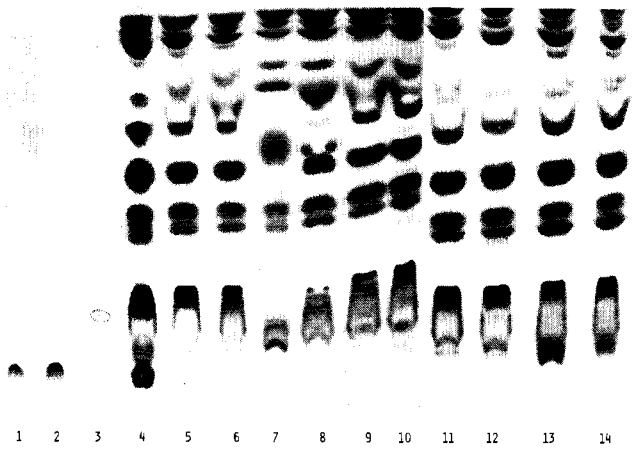

Figure 1. Lysinoalanine fractionation on cellulose TLC sheets (Eastman No. 6064 without fluorescent indicator) in protein hydrolysates from canned baby foods. (1) Lysinoalanine, 0.1 μg; (2) lysinoalanine, 0.2 μg; (3) D-(+)-galactosamine, 0.5 μg; (4) alkali-treated albumin; (5) baby food no. 4, unheated; (6) baby food no. 1, heated; (7) baby food no. 4, unheated; (8) baby food no. 4, heated; (9) baby food no. 5, unheated; (10) baby food no. 5, heated; (11) baby food no. 3, unheated; (12) baby food no. 3, heated; (13) baby food no. 2, unheated; and (14) baby food no. 2, heated. Spots 11–14 are from a separate developing.

observations presented by Rakowska (1972), who reported losses of 15–20% of the available lysine and NPU values in some canned baby foods.

Figure 1 shows the results of a lysinoalanine assay run on some of the baby food samples mentioned above. As can be seen from this figure, only traces of lysinoalanine were found in heat-treated samples. Alkali-treated albumin, however, gives a strong spot compared to the baby food samples. We figured out that lysinoalanine content is much less than 0.1 mg/g of protein, and thus much lower than toxic levels presented by Sternberg et al. (1975a).

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Gas–Liquid Chromatographic Analysis of Carbaryl as Its *N*-Thiomethyl Derivative

N-Thiomethyl- and *N*-thio-*p*-tolylcarbaryl were prepared, and a residue analytical procedure was developed for carbaryl using methylsulfenyl chloride as the derivatizing agent and using gas chromatography with a flame photometric detector operating in the sulfur-selective mode. The method was applied to beans, carrots, lettuce, and tomatoes fortified at the 5 and 10 ppm levels.

The thermal instability of the *N*-methylcarbamate insecticides has hampered the application of gas chromatographic techniques to the direct determination of their residues. One approach to the problem has been to replace the N-H proton with an organic moiety which would confer thermal stability to the carbamate. This derivatization step also offers an opportunity of adding a moiety which enhances the detectability of the carbamate to specific detectors such as the electron-capture or microcoulometric detectors. N derivatives that have been investigated for residue analytical purposes are *N*-trimethylsilyl (Fishbein and Zielinski, 1965), *N*-acetyl (Epstein et al., 1967; Sullivan et al., 1967; Magallona and Gunther, 1974), *N*-trifluoroacetyl (Lau and Marxmiller, 1970), *N*-pentafluoropropionyl and *N*-heptafluorobutyryl (Seiber, 1972), and the *N*-mono-, di-, and trichloroacetyl derivatives (Magallona and Gunther, 1977).

Westlake et al. (1972) used a flame photometric detector (FPD) in the sulfur mode to analyze for RE-11775 (*m*-*sec*-butylphenyl *N*-methyl-*N*-thiophenylcarbamate). Since sulfur allows the use of an element-specific detector and therefore some degree of selectivity over background interferences, the possibility of utilizing methylsulfenyl and *p*-toluenesulfenyl chloride to prepare *N*-thiomethyl and *N*-thio-*p*-tolyl derivatives of *N*-methylcarbamates for residue analytical purposes was investigated. Carbaryl (1-naphthyl *N*-methylcarbamate) was used as a model compound.

EXPERIMENTAL SECTION

Synthesis. *Methylsulfenyl Chloride.* A round-bottom flask containing 50 mmol of dimethyl disulfide was equipped with a magnetic stirrer bar, condenser, and dropping funnel containing 50 mmol of sulfuryl chloride. With the flask cooled to about −10 °C, one-half the SO_2Cl_2 was added dropwise to the stirred dimethyl disulfide. The temperature was allowed to rise slowly to room temperature and the remainder of the SO_2Cl_2 was added. The

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