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Analysis of diacetyl in yogurt by two new spectrophotometric and fluorimetric methods

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Two new methods (spectrophotometric and fluorimetric) are reported for the determination of diacetyl in yogurt. The diacetyl was isolated by distillation and condensed with isoniazide. This solution was then treated with Zr(IV) to form a complex, which was measured with these two methods. Average recovery of diacetyl was 96·8% for spectrophotometry and 99·1% for fluorimetry, while precision was RSD = 2·5 and 1·0%, respectively. The concentration of diacetyl in yogurt ranged from 0·79 to 2·77 μg/g. The two procedures gave statistically similar results. The fluorimetric method was compared with a traditional method (gas chromatography), for which acetaldehyde, acetone, methanol, and ethanol were also determined.

INTRODUCTION

Yogurt is the product of milk fermentation by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, and these bacteria are also the main production source of the aromatic compounds in yogurt. Among these compounds are non-volatile acids (lactic, pyruvic, etc.), volatile acids (formic, acetic, propionic, etc.), carbonilic compounds (acetaldehyde, acetone, acetoin, diacetyl, etc.), and a heterogenous group of substances formed during the thermal degradation of proteins, fats and lactose (Tamime & Robinson, 1990). Diacetyl, lactic acid and acetaldehyde contribute most to the final flavour, and their concentration and relative levels determine the 'quality' of the product and its acceptance by consumers (Lindsay *et al.*, 1965; Bottazzi & Dellaglio, 1967).

Traditionally, these compounds have been determined in yogurt and milk cultures employing gas chromatography, using either normal (Hild, 1980; Degorce-Dumas *et al.*, 1986; Kang *et al.*, 1988) or capillary columns (Montville *et al.*, 1987). Colorimetric techniques have also been applied, particularly with diacetyl (Walsh & Cogan, 1974; Hegazi & Abo-Elgana, 1989; Vilchez *et al.*, 1990), and recently an HPLC method has been used (Matsuura *et al.*, 1990).

The diacetyl content in yogurt has been determined using three methods: colorimetric (spectrophotometry and fluorimetry) and gas chromatography with flame ionization detector and capillary column. The latter has also been applied to the determination of other volatile flavour compounds, particularly acetaldehyde, acetone and ethanol.

EXPERIMENTAL

Apparatus

The following equipment was used: a 26 pH-meter Radiometer with glass and calomel electrodes; a Perkin–Elmer 551-S UV-VIS spectrophotometer equipped with 1 cm quartz cells and 1 nm slit width; a Perkin–Elmer 204 spectro-fluorimeter equipped with a Xenon lamp, 10 × 10 mm quartz cells, and 10 nm slit width, at room temperature.

A Perkin–Elmer Sigma 3B gas chromatograph was used, equipped with a flame-ionization detector. The column was a fused silice Carbowax 20 M column (50 m × 0·25 mm). Operating conditions were as follows: injector 150°C, detector 150°C, and column temperature 70°C; carrier gas (nitrogen) flow rate 1·20 ml min^{-1}, split ratio 1:20, injection volume 0·5 μl. The chromatograms were recorded on a Perkin–Elmer model LCI 100 data processor.

Reagents and samples

Analytical-grade reagent chemicals were used. Thirty samples of yogurt from several different brands were analysed. They were bought at supermarkets and consist of eight distinct types. Diacetyl content was determined in the samples with spectrophotometry and fluorimetry. Eight samples of plain yogurt were analysed via gas chromatography and fluorimetry.

METHODS

Spectrophotometric and fluorimetric methods

Two methods developed in the authors' department were used for the analyses, one spectrophotometric (García-Villanova & García Estepa, 1993*a*) and the other fluorimetric (García-Villanova & García Estepa, 1993*b*). These methods are based on the condensation of diacetyl with isoniazide (INH) in an acetic acid (HAc) medium and the subsequent formation of a complex with Zr(IV) in a highly acidic medium.

Samples of 25·0 g (spectrophotometry) or 2·5 g (fluorimetry) were mixed with 70 ml of a NaCl-saturated solution. The mixture was distilled until some 30 ml was collected in an INH solution acidified with acetic acid. The reaction continues according to the conditions described in Table 1. Final volume was 50 ml.

Spectrophotometry

Beer–Lambert's law holds for diacetyl concentrations of between $0{\cdot}29 \times 10^{-5}$ M and $5{\cdot}8 \times 10^{-5}$ M ($r = 0{\cdot}9999$ and $\epsilon = 15{\cdot}5 \times 10^3$ l/mol·cm).

Fluorimetry

The Fluorescence–Intensity–Concentration relationship is lineal in the concentration region lying between $2{\cdot}32 \times 10^{-7}$ M and $2{\cdot}32 \times 10^{-6}$ M ($r = 0{\cdot}9998$).

Chromatographic method

NaCl-saturated solution (75 ml) is added to 75·0 g of yogurt in a 250-ml destilling matrass. The sample was distilled until 25 ml of distillate was collected in a volumetric flask in an ice bath; 1 ml of 200 ppm isopropyl acetate solution (internal standard) was added to 9 ml of the destillate, and 0·5 μl of this solution was then injected.

Identification of the components was made by adding a standard solution of the compound to be identified and using the TRR of the internal standard (isopropyl acetate). Figure 1 shows a type-chromatogram; as may be seen, other components besides the diacetyl (5) were able to be determined: acetaldehyde (1) acetone (2), methanol (3) and ethanol (4).

The statistical analysis to obtain the linear-regression equations was performed using the biostatistical data base Sigma, supplied by Horus Hardware S.A., Madrid, Spain. The component dissolution concentrations and the area of the peaks obtained were considered as the variables for the equations.

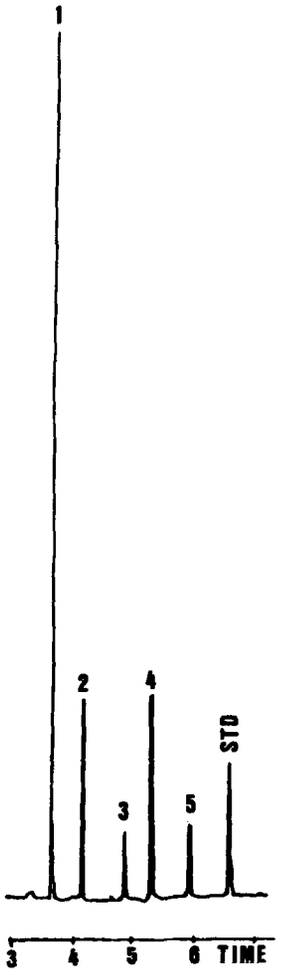

Fig. 1. Typical chromatogram of aromatic compounds in yogurt.

RESULTS AND DISCUSSION

The accuracy of the fluorimetric and spectrophotometric methods was tested with recovery assays. Increasing quantities of a standard solution of diacetyl were added to the sample and recovery was carried out as described above (see Table 2 for results).

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Table 1. Reaction conditions and analytical characteristics of spectrophotometric and fluorimetric methods

Reaction conditions	Spectrophotometry	Fluorimetry
Isoniazide added to	Excess 300	Excess 1500
Time	10 min	15 min
Temperature	Room temperature	Room temperature
Zr(IV) added to	Excess 50	Excess 2500
Time	30 min	30 min
Temperature	Room temperature	Room temperature
HCl added to pH	1·7 ± 0·1	1·7 ± 0·1
Wavelengths	410 nm	Excitation 410 nm Emission 510 nm
Blank	INH+HAc+Zr(IV)+HCl	INH+HAc+Zr(IV)+HCl

increasing quantities of diacetyl standard solution, increasing quantities of standard solutions of acetaldehyde, acetone, methanol, ethanol and propanol were added to the sample and recovery was carried out as described above (see Table 3 for results).

The recovery range for diacetyl was 96·2–97·8% by the spectrophotometric method ($\bar{x}$ = 96·83%), 97·7–100·9% by the fluorimetric method ($\bar{x}$ = 99·13%) and 91·1–93·4% by the chromatographic method ($\bar{x}$ = 91·8%).

Recovery was over 90% in all cases, although the fluorimetric method gave the results closest to 100%.

The other aromatic compounds analysed by gas chromatography also had very high recovery rates, averaging 97·7% for acetaldehyde, 99·4% for acetone, 98·27% for methanol, 94·87% for ethanol and 93·67% for propanol.

Table 2. Diacetyl recovery by spectrophotometric and fluorimetric methods

Method	Added (μg/g)	Total (μg/g)	Found (μg/g)	Recovery[a] (%)
Spectrophotometric[b]	5·0	13·7	13·4	97·8
	10·0	18·7	18·0	96·2
	15·0	23·7	23·0	97·0
	20·0	28·7	27·6	96·2
Fluorimetric[c]	0·40	1·72	1·68	97·7
	0·80	2·12	2·14	101
	1·20	2·52	2·14	100
	1·60	2·92	2·86	97·9

[a]Mean of three determinations.
[b]Yogurt contained 1·74 μg/g diacetyl.
[c]Yogurt contained 1·32 μg/g diacetyl.

Table 3. Study of aromatic-compound recovery in yogurt using GLC[a]

Compound	Added (μg/g)	Total (μg/g)	Found (μg/g)	Recovery[b] (%)
Acetaldehyde	5·0	40·3	39·0	96·8
	15·0	50·3	45·3	90·0
	30·0	65·3	65·5	100
Acetone	0·75	2·68	2·58	96·3
	1·50	3·43	3·36	98·0
	3·00	4·93	5·12	104
Diacetyl	0·75	2·13	1·94	91·1
	1·50	2·88	2·62	90·9
	3·00	4·38	4·09	93·4
Ethanol	5·00	9·10	8·19	90·0
	10·0	14·1	13·6	96·6
	15·0	19·1	18·7	98·0
Methanol	1·50	3·56	3·48	97·8
	3·00	5·06	5·16	102
	6·00	8·06	7·66	95·0
Propanol	0·75	2·11	1·98	93·8
	1·50	2·86	2·69	94·1
	3·00	4·36	4·06	93·1

[a]Yogurt contained 35·29 μg/g acetaldehyde, 1·93 μg/g acetone, 1·38 μg/g diacetyl, 4·10 μg/g ethanol, 2·06 μg/g methanol and 1·36 μg/g propanol.
[b] Mean of three determinations.

Ten determinations were made to determine accuracy. Results for diacetyl (by the three methods tested), as well as for the rest of the compounds, were satisfactory (with the exception of propanol) (Table 4).

Table 4. Precision study

Method	Compound	Concentration (μg/g)	RSD[a] (%)
Spectrophotometric	Diacetyl	1·55	2·50
Fluorimetric	Diacetyl	1·55	1·02
Chromatographic	Diacetyl	1·38	2·20
	Acetaldehyde	35·3	1·35
	Acetone	1·93	2·01
	Ethanol	4·10	3·19
	Methanol	2·80	1·59
	Propanol	1·36	9·19

[a]Mean for 10 determinations.

Table 5. Diacetyl content of yogurts

	Methods	
Type	Spectrophotometric (μg/g)	Fluorimetric (μg/g)
Plain		
1	1·49	1·53
2	1·16	1·15
3	2·45	2·39
Plain sweetened		
4	1·05	0·99
5	2·33	2·31
Extra creamy		
6	1·05	1·09
7	1·64	1·67
8	1·31	1·27
Skimmed		
9	1·82	1·83
10	1·64	1·59
11	1·99	1·59
12	1·82	1·64
13	2·07	2·17
14	1·51	1·43
Flavoured		
15	1·99	1·98
16	2·33	2·31
17	1·51	1·43
18	2·61	2·53
19	1·05	1·07
20	1·03	1·09
With fruit		
21	1·64	1·63
22	1·60	1·67
23	2·77	2·71
Bifidus		
24	2·77	2·71
25	2·49	2·51
Liquid		
26	0·79	0·87
27	0·87	0·95
28	0·79	0·89
Imitation		
29	1·16	1·25
30	1·36	1·43

Thirty yogurt samples from eight brands commercialised in Spain have been analysed. Table 5 lists the mean diacetyl content obtained by spectrophotometric and fluorimetric methods.

The two methods were statistically compared using the biostatistical data base Sigma. No statistical differences were found ($P < 0.928$), although the fluorimetric method is much more sensitive and therefore requires much smaller quantities of sample.

Concentration range was 0·79–2·77 μg/g, values that are similar to those obtained by McGregor and White (1987), higher than those of Degorce-Dumas *et al.* (1986) and of Murti *et al.* (1993), and much lower than those obtained by Hild (1980). In the present samples, the highest levels of diacetyl were determined for *Bifidus* yogurt and the lowest for liquid yogurts.

The fluorimetric method proposed here was compared with the more traditional gas chromatography for diacetyl determinations. Eight samples of plain yogurt were analysed (see Table 6) and the methods were statistically compared using the biostatistical data base Sigma mentioned above. No significant differences were found ($P < 0.937$).

The minimum amount of yogurt needed for the chromatographic determinations, while similar to that needed for the spectrophotometric method, was much greater than is used in the fluorimetric technique. Nevertheless, it must be recognized that gas chromatography allows other aromatic volatile compounds in the yogurt distillate to be determined (e.g. acetaldehyde, acetone, methanol, ethanol and propanol) (Table 7).

Table 6. Diacetyl content in plain yogurt analysed by fluorimetry and chromatography

Sample	Methods	
	Fluorimetric (μg/g)	Chromatographic (μg/g)
1	1·67	1·38
2	1·53	1·89
3	0·91	1·15
4	2·17	2·16
5	1·03	0·86
6	1·97	2·07
7	2·95	3·17
8	2·71	2·50

Table 7. Acetaldehyde, acetone, methanol, ethanol and propanol content (μg/g) in plain yogurt

Sample	Acetaldehyde	Acetone	Methanol	Ethanol	Propanol
1	35·3	1·93	2·80	4·10	1·36
2	38·2	2·00	4·86	3·38	—
3	26·6	5·65	—	9·05	—
4	24·2	3·25	—	9·75	1·87
5	23·5	2·36	—	3·67	—
6	43·8	3·71	2·06	4·03	—
7	11·6	2·62	—	7·55	1·87
8	8·2	2·65	—	6·82	—

As expected, the main component found was acetaldehyde, the principal source of yogurt flavour. Concentration ranged from 8·16 to 43·78 μg/g, with an average of 25·15 μg/g. These results are in agreement with Murti *et al.* (1993) and McGregor and White (1987) but are higher than those obtained by Degorce-Dumas *et al.* (1986) and Hild (1980). The last two authors also found slightly lower amounts of acetone and ethanol than the present authors did. Methanol and propanol were detected in three of our samples, although the bibliography makes no mention of their existence in yogurt. The peaks were identified and determined as such based on their TRR coinciding with the internal standard compounds.

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