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Carbon dioxide measurement in Swiss-type cheeses by coupling extraction and gas chromatography

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Summary — The microbial production of carbon dioxide (CO_2) in Swiss-type cheeses is one of the major events during ripening. To better control this formation of eyes, it is necessary to be able to evaluate the CO_2 in the body of the cheese. In this paper, we propose a method to liberate the CO_2 from the cheese sample dispersed in a citric alkaline solution in a blender and to quantify the liberated gas, by acidification, by the direct coupling between the extraction and a gas chromatography analyser. The proposed method has a linearity between 0 to 10 mmol l^{-1} of CO_2, a coefficient of variation of 1.20% and a good repeatability.

carbon dioxide / cheese / gas chromatography / analytical method

Résumé — Détermination de la teneur en dioxyde de carbone des fromages de type Suisse par couplage extraction-chromatographie en phase gazeuse. *La formation du gaz carbonique (CO_2) au sein des fromages à pâte pressée cuite est l'un des événements majeurs observables au cours de l'affinage. Le strict contrôle de la formation des yeux nécessite une méthode fiable de mesure du CO_2 dissous dans la pâte. Dans cet article, nous décrivons une méthode qui permet d'extraire le CO_2 d'échantillons de fromage broyés dans une solution citrique alcaline et d'en mesurer la quantité à l'aide d'un chromatographe en phase gazeuse après acidification de la solution. La méthode proposée présente une parfaite linéarité de réponse entre 0 et 10 mmol l^{-1} de CO_2, un coefficient de variation de 1,20% et une bonne répétabilité. De plus, elle est simple à mettre en œuvre.*

dioxyde de carbone / fromage / chromatographie en phase gazeuse / méthode d'analyse

INTRODUCTION

In France, the production of Emmental cheese has reached 212 000 tons in 1993 (CNIEL, 1994). This hard cooked cheese is the first French cheese and the second, on a world-wide scale, after Cheddar. In this kind of Swiss-type cheese, production of carbon dioxide (CO_2), due to lactate breakdown by propionibacteria (Steffen *et al*, 1987), plays one of the major roles during the ripening process by allowing eyes formation. In the cheese loaf, the amount of CO_2 is scattered as follows: 48% dissolved in the body, 20% in the eyes and 32% diffused through the rind (Steffen *et al*, 1987). In order to optimise the maturation process and so the quality of cheese, it is necessary to measure the CO_2 dissolved in the body all along the cheese processing.

In the literature, most of the data concerning CO_2 produced by propionibacteria in a culture media or related to the bacterial metabolism in cheese, are calculated from the final products or intermediate compound concentrations and from one or several equations reflecting roughly the carbon metabolism (Hettinga and Reinbold, 1972). Even using the work done by Wood (1981) on propionibacteria, these sketchy data do not permit to construct an accurate carbon balance. Moreover, none was carried out using lactate as a substrate. CO_2 measurements are always failing to reach the 100% carbon recovery.

The first attempt to measure CO_2 generated by micro-organisms was performed by Leichman (1896). Then, different well-known authors, generally working on lactic acid bacteria (Freudenreich and Orla-Jensen, 1906; Orla-Jensen, 1919; Allen, 1939), developed special apparatuses, but none of them appeared reliable.

In 1945, Gibson and Abdel-Malek measured CO_2 production by incubating cultures in a volumetric flask with a calibrated neck. This method was used to differentiate heterofermentative lactic acid bacteria, organisms producing a detectable quantity of gas, from other lactic acid bacteria or spore-forming bacilli. They demonstrated that the amount of CO_2 formed during fermentation of sugars depends on several factors such as sugar (nature and concentration) and buffering capacity of the medium. Therefore, this method was not quantitative. Gravimetric, manometric and titrimetric methods were used until 1978 (Sandine *et al*, 1957; Robertson, 1957, 1962; Hoglund *et al*, 1972; Flückiger *et al*, 1978). More recently, Bosset *et al* (1986) measured the partial pressure of CO_2 in various liquid fermented milk products using a gas-sensitive CO_2 electrode. In a review article, Dixon and Kell (1989) described more than fifteen methods used in estimation of determination of CO_2 during fermentations, in the liquid or gas phase. In 1992, a simple method based on the chemical reaction of CO_2 with a specific indicator layer packed into gas diffusion tubes was described by Kneifel and Gretner. Mohr *et al* (1993) adapted the method of King and Mabbitt (1982) who used gas chromatography to measure CO_2 that was added to milk in order to preserve it, by lowering the pH. Gas chromatographic methods were previously developed (Kreula and Moisio, 1970; Ross, 1987) but required a sample preparation of 17 to 24 h, neglected the solubility of CO_2 in aqueous solutions and did not account for the difference of pressure among the bottles.

Data on the level of CO_2 dissolved in the body of hard-cooked cheese, important during ripening, are not available in the factories, probably because of the difficulties encountered to measure it. Therefore, attempts were made to develop methods in the beginning of the century (Clark, 1917). Hoestettler (1944) estimated the CO_2 in Emmental cheese by drawing the gas off under vacuum. Hiscox *et al* (1941) estimated the dissolved CO_2 of Cheddar cheese in connexion with the estimation of volatile acids by subtraction of values obtained with aerated samples from the values obtained with non-aerated samples. In 1953, Swartling and Willart, working on Herrgärt cheese, developed a titrimetric method. The sample was emulsified in alkali, acidified and CO_2 was carried away in CO_2-free air from which it was absorbed in a standard solution of barium hydroxide. The excess of barium hydroxide was measured using titrated acid. Robertson (1957, 1962) adapted the previous method to measure the CO_2 content of New-Zealand Cheddar cheese. Later on, in the eighties, a Swiss team (Bosset *et al*, 1980) carried on with a new method to determine CO_2 in food products with particular application to cheese. Samples were emulsified, under reduced pressure, in an alkaline citrate medium using a mixer. Carbon dioxide was quantitatively displaced into the headspace by addition of an excess of sulphuric acid. Measurements were carried out with a specific non-dispersive infrared detector. Afterwards, Bosset *et al* (1989) described an analyser based on gas chromatographic separation with thermal conductivity detection which can be used for the analysis of gas atmosphere over dairy products such as canned milk powder, canned condensed milk, cheese packed in cans or with plastic film and yogurt. An enzymic procedure was used for determining CO_2 in blood serum (Forrester *et al*, 1976). The procedure was adapted by Crow and Martley (1991) for the measurement of CO_2 dissolved in Cheddar cheese. But none of these methods can be routinely used. Titrimetric methods are not accurate (10%). Enzymic techniques are much more accurate (0.4%) however, such a kind of cheese as Emmental shows marked variations of CO_2 concentration within the loaf of cheese so the sample (5 g) becomes absolutely not representative. Methods using infrared detector require, like titrimetric methods, the release and measurement of the total amount of CO_2 (accuracy: 2%). The vapor must be maintained constant because of its interference with the measurements.

The aim of the present investigation was to determine CO_2 dissolved in Swiss-type cheese. The method was chosen, taking into account the different methods already studied. CO_2 was estimated using a gas/solid chromatography analyser equipped with a thermal conductivity detector, after treatment of the sample according to the method of Bosset *et al* (1979, 1980).

MATERIALS AND METHODS

Analytical system

The analytic system is illustrated in figure 1. The sample was introduced in a modified stainless steel gas proof 1-l blender (Waring S 1021, Standa Industrie, Caen, France) equipped with a two-speed motor. The cover was modified by welding five connexions (5 cm long and a 5 mm diameter). The first one was equipped with a Bourdon depression-meter (0 to –760 mbar, Hartereau, Rennes, France). The second one was connected to the controlled vacuum system. The third one was connected to the reference gas (CO_2 1% ± 0.02, v/v, in nitrogen, Aga, France). The fourth one was connected to the gas chromatograph and the last one was equipped with a teflon funnel. CO_2 was analyzed in a Varian gas chromatograph (3400, equipped with a 250 μl automatic injection loop, Les Ulis, France). Separation took place in a packed column (3.66 m x 3.2 mm; Haye Sep, N,80/100 Mesh) at 105°C with highly pure hydrogen as gas vector (N55, Alphagaz, France, flow rate 50 ml^{-1}, pressure 2.8 x 10^5 Pa. A thermal conductivity detector was used at 140°C. The results were analyzed and stored in a microcomputer equipped with the software Gold (Beckman, USA). Each analysis took 4.5 min. Between each sample, 10 min were required in order to clean the column (at 140°C).

Sampling and sample preparation

Each piece of cheese was a 3 kg parallelepipedic block of 9 cm width, with both rinds, taken in the

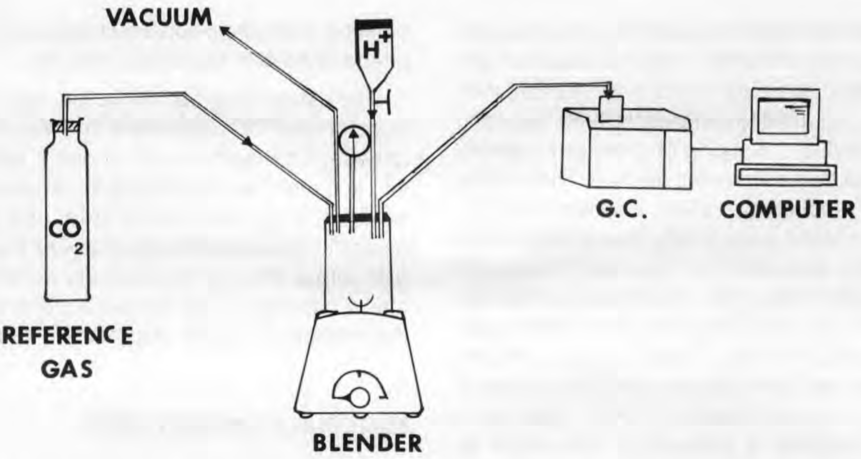

Fig 1. Equipment used for the determination of CO_2 in cheese.
Équipement mis en œuvre pour la mesure du CO_2 dans les fromages.

central part of the loaf, kept in a sealed bag, under low pressure, at + 4°C. Six to eight samples from 30 to 50 g were collected in each block with a cheese borer (1.8 x 18 cm, in stainless steel).

The sample was then immersed and finely dispersed during storage in 60 g of a citric alkaline solution (weighted exactly) (sodium hydroxide in pellets, 10 g l^{-1} (Merck, pro analysi, Darmstadt, Germany) and tri-sodium citrate, 50 g l^{-1} (Merck, Germany), dissolved in a freshly distilled, sterilized water, cooled under low pressure to avoid air redissolution) and stored in gas-proof vials. Just before analysis, water was added to the sample in order to supplement the cheese weight to 100 g (to avoid variations of volume in the blender; Bosset *et al,* 1980).

Analysis

The sample was then placed in the blender, which was subsequently connected to vacuum (–450 ± 50 mbar). The blender was then isolated; the grinding was performed under partial vacuum for 2 min. After 1.5 min, 50 ml of sulfuric acid (2.5 mol l^{-1}, Merck, Darmstad, Germany) was added from the teflon funnel. The blender was gently turned back to atmospheric pressure then isolated before several automatic injections were finally carried out.

The four first ones were used to drain the 1.0 ml volume of the tubing between the blender and the injection loop and discarded. The four next ones were then collected and the value was the arithmetic mean of these four injections.

The result was then calculated from the following equation:

$$\frac{\%\ CO_2 \times (\text{vol (blender)-vol (cheese solution)}) \times 1000}{100 \times 25220 \times \text{mass of cheese (g)}} = y \text{ mol } CO_2\text{/kg of cheese}$$

with vol = volume

R = 25.22 ml CO_2/mmol CO_2 at 20°C and under 720 mbar.

Calibration of the analytic system

This procedure was done weekly. The stability of this calibration along this period has been verified. In order to discard completely the air of the blender, vacuum was firstly applied (–700 mbar) and then highly pure CO_2 (1%, v/v) was added in it, until atmospheric pressure was restored. This step was repeated three times. Afterwards, a continuous CO_2 flow rate was allowed to sweep the blender to be sure to maintain CO_2 at the atmospheric pressure (otherwise, an overpression can alter the quality of the calibration) and that the multiple injections did not create any depression also detrimental for the analysis. Nine injections were then carried out in which the five last were taken into account to calibrate the apparatus. Six calibrations were performed (standard deviations: 0.025 for CO_2 at 1%).

Linearity of the method

In order to evaluate the linearity of the method, solutions of sodium carbonate 114 mmol l^{-1} and 228 mmol l^{-1} (Merck, Darmstadt, Germany) were used to liberate CO_2 in the blender by addition of sulphuric acid (same procedure as for samples):

$$Na_2CO_3 + H_2SO_4 \longrightarrow H_2O + CO_2 \nearrow + Na_2SO_4$$

RESULTS

Limit of detection

The limit of detection was calculated at 6.3 10^{-3} mmol l^{-1} of CO_2 (0.016%) on the base of a signal-to-noise ratio of 3 (from a chromatogram of CO_2 at 1% in nitrogen). The CO_2 content of the air of the laboratory was measured. A set of values of 0.040% ± 0.010 was easily detected. This value is in close agreement with the value of 0.031% ± 0.005 of the literature (Kuiperg, 1976). It was not possible to test lower concentrations as calibrated gas was not available below this limit. Lower gas concentration could be prepared by dilution of the reference gas. But, as already demonstrated by Bosset *et al* (1989), the biggest error will be in the preparation of the diluted gas rather than in the measurement itself.

Linearity of the method

Figure 2 illustrates the response of the complete device to the liberation of CO_2 from sodium carbonate. The regression line can be described by the equation:

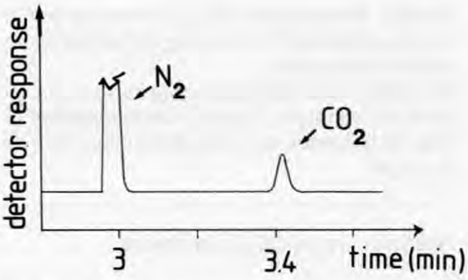

Fig 2. Example of a chromatogram obtained by this method.
Exemple de chromatogramme obtenu à l'aide de cette méthode.

$$\text{peak area} = 0.64 \times \text{mmol } CO_2 + 0.12$$

(with $r = 0.998$)

which is usable for the conversion of peak area in content of carbon dioxide in the sample (before data reduction to 1 kg sample weight). The method has a good linearity between 0 to 10 mmol l^{-1} of CO_2.

Repeatability of the method

In order to analyse this characteristic, 330 ± 20 g of a cheese block were prepared in the citric alkaline solution kept in a gas-tight glass bottle. Six series of analysis were performed with this preparation. The results are presented in table I. The mean value is 24.1 mmol of CO_2 for 1 kg of cheese. The coefficient of variation (CV) is 1.2%.

The application of such a method to the determination of the carbon dioxide content of cheese samples needs a blank which must be prepared and measured before each cheese sample (especially if the value of this blank and of the sample are time-dependent as is the case in this type of measurement). Moreover, the stability of the gas content of the sample between sampling and measurement must be ensured in order to present an interest for the professionals.

Table I. Repeatability of CO_2 measurement in cheese. Example for six analyses of the same cheese preparation.
Évaluation de la répétabilité de la mesure de CO_2 dans les fromages. Exemple de la répétition (6 fois) de la mesure sur une même préparation de fromage.

Analysis	*mmol CO_2/kg of cheese*	
1	24.5	mean (X) = 24.1
2	23.8	SD = 2.9
3	24.2	CV = 1.20%
4	23.6	
5	24.2	
6	24.1	

The blank contains all the constituents except the cheese. The value of this blank has been systematically subtracted from the value determined with the sample.

Importance of the gas tightness of the bottles used for the storage

When the plastic sampling bottles were used to keep the citric alkaline solution alone, the results were systematically higher than for the samples kept in glass bottles, leading to errors from 2 to 15% (table II). Cheese solutions kept in plastic bottles were not stable in term of CO_2 content. These increasing values are mainly due to the carbonatation of

Table II. Influence of storage time and storage conditions (vials) between the immersion in the citric alkaline solution and analysis.
Influence du temps de conservation et des conditions de conservation de l'échantillon dans la solution citrique alcaline avant analyse.

Day of storage	*mmol of CO_2/kg of cheese*						
	Glass bottle				*Plastic bottle*		
	Sample 1	*Sample 2*	*Blank*	Δ	*Sample 3*	*Blank*	Δ
0	18.0	20.4	1.6	16.4; 18.8	21.5	1.6	19.9
	17.1			15.5	21.6	t + 6 h = 2.0	20.0
1	ND	20.5	1.6	18.9	ND	ND	
2	18.0	ND	1.9	16.1	22.3	2.3	20.0
	1.78			15.9	24.4		22.1
3	ND	20.5	1.8	18.7	ND	ND	
		20.9		19.1			
4	17.9	ND	1.7	16.2	23.1	2.0	21.1
	17.7			16.0	23.1		
7	17.9	21.0	1.6	16.3	2.28	2.8	20.0
				19.4	2.47		21.9

ND: not determined. Blank = citric alkaline solution without cheese. Δ = sample value – blank value. Some trials have been performed twice (two values).
ND : non déterminé. Blank = solution citrique alcaline sans échantillon de fromage. Δ = valeur de l'échantillon après soustraction du témoin. Certains échantillons ont fait l'objet de 2 séries de dosages conduisant à 2 valeurs.

the citric alkaline solution through the container: 0.16 to 0.28 mmol l^{-1} CO_2 in 7 days of storage (table II). On the opposite, when the samples were kept in glass bottles, we observed no difference in CO_2 measurements during 7 days of storage (table II).

Sampling

The losses of CO_2 between the cheese samples extracted from the loaf are fast and considerable due to the acidic medium of cheese body ($H_2CO_3 \longrightarrow H_2O + CO_2$) and to the difference of partial pressure of CO_2 between cheese body and ambient air. They lead to a substantial decrease of the CO_2 value. In order to show this phenomenon, several cheese bores were not immediately immersed in the citric alkaline solution but let in the laboratory atmosphere 12 h before dispersion/dissolution treatment. The normally treated samples (of the same cheese block) presented 31.0 mmol CO_2/kg of cheese (CV: 3.0%) while the other one presented only 8.7 mmol CO_2/kg of cheese.

This CV of 3.7% is high in comparison with the CV of 1.2% of the method. It is probably due to the difference of the surface exchange cheese-air between the different samples (eyes number, size and heterogeneity of the samples).

Heterogeneity of the CO_2 content of the cheese

The heterogeneity of the CO_2 content within a cheese block is evidenced in table III. The CV varied from 0.9 to 8.1%, value higher than the 1.2% of the repeated analysis of the same cheese preparation (table I). These variations in the measurement of the CO_2 content are due much more to sample heterogeneity than to the repeatability of the method.

Table III. Heterogeneity of the CO_2 content of the cheese body.
Hétérogénéité du CO_2 dissous dans la pâte.

Samples	*X mmol/kg of cheese*	*SD*	*CV (%)*	n
A	23.3	1.2	5.1	6
B	22.6	1.8	7.9	5
C	24.9	0.9	3.6	6
D	23.3	1.9	8.1	5
E	25.0	0.9	3.6	6
F	31.0	0.3	0.9	3

Each value is the average of 3–6 measurements for the same piece of cheese. The samples were taken from different cheeses (A–F).
Chaque échantillon est caractérisé par sa moyenne X, son écart type (SD) et son coefficient de variation (CV) calculé sur la base de 3, 5 ou 6 mesures (n) effectuées sur la même meule de fromage. Les échantillons A à F proviennent de meules différentes de la même sorte de fromage (Swiss-type).

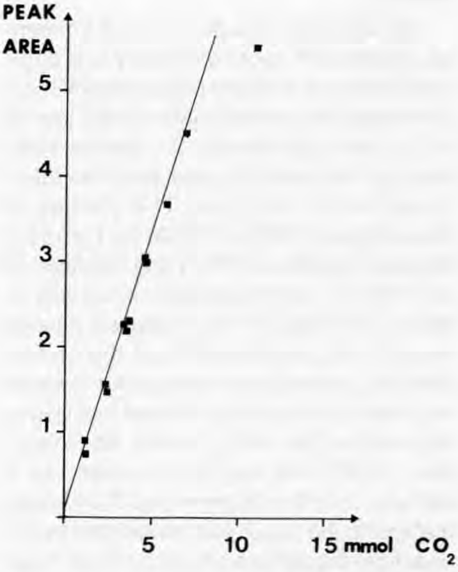

Fig 3. A standard curve of gas chromatographic peak areas of CO_2 derived from solutions of sodium bicarbonate of different molarities.
Un exemple des courbes d'étalonnage obtenues avec des solutions de bicarbonate de différentes molarités.

DISCUSSION

The proposed method can be compared with the others already published in the literature for CO_2 measurements in cheese. This method used a detector which has been proven to present an excellent linear response from 0 to 10 mmol l^{-1} of CO_2 with a high sensitivity and a detection limit of 6.3 10^{-3} mmol l^{-1}. Bosset *et al* (1980) presented a CV of 1.66% (here 1.2%; $n = 6$) with a standard deviation of 0.0934 ($n = 10$) (here 0.029 which is comparable to the value 0.033 ($n = 3$) obtained by Hoglund *et al*, 1972). The standard deviation of the enzymatic method was 1 mmol/kg of cheese (mean value: 11 mmol/kg; $n = 6$) for estimation of CO_2 in Cheddar cheese (Crow and Martley, 1991). The gas chromatographic technique developed by Ross (1987) had a CV of 8.0% ($n = 3$) for within-day variation.

The sampling from 30 to 50 g of Emmental cheese with a cheese borer is a common practice in industry and is already used to evaluate the eyes formation in the loaves and to taste the cheese. So, cheese sampling by this method could easily be introduced in the factories. The studies of Swartling and Willart (1953) for Herrgård cheese, Robertson (1957) and Flückiger *et al* (1978) for Swiss-type cheeses as well as Blanc *et al* (1980) for Swiss Gruyère cheese have clearly established that the carbon dioxide concentration decreased towards the outer surface of the cheese loaf due to its pk value (pka of $CO_2 = 4.6$). So Robertson (1957) had decided to take, as a sample, only the deepest end of the plug, discarding the upper part considered as too near from the surface and so having a lower CO_2 content than the inner part of the cheese. This concentration gradient of carbon dioxide is not the only reason of the heterogeneity of the sample. The cheese borer does not allow to take sample with 'complete eye'. Most of the eyes are cut through, so their particular gas content is immediately lost into the laboratory atmosphere. The cheese body surrounding the eyes has probably a slightly different CO_2 content than the rest of the body. So, the density of the eyes in the bore is probably of importance in the CO_2 measurement.

Based on the results of Bosset *et al* (1980) and our own results, we proposed to immediately immerse and weigh this sample, in a citric alkaline solution prepared in a gas proof glass bottle. This bottle can be stored and subsequently analysed or mailed to the central laboratory for measurement without any problem for several weeks.

This effect of carbonatation of sodium hydroxide is well known but its importance is particularly evidenced when low levels of CO_2 must be measured as is the case in our method. Moreover, the preparation of this alkaline solution must be done with freshly distilled water and well protected caustic tablets. In a proper gas proof glass container, this solution can be kept 2 weeks. The only possibility to take into account this interference effect is to determine and subtract the corresponding blank. This method allows the investigation of carbon dioxide formation in hard and semi-hard cheeses like Emmental, but also for cheeses with lower eyes formation (Comté, Gouda, Mimolette). This information is of great interest to follow the propionic acid fermentation in such cheeses.

This method presents some limitations: only four samples can be measured per hour and the analysis must be performed by well-trained technicians. In order to increase the quality of the measurements day-to-day temperature and atmospheric pressure variations should be taken into account.

The elaboration of a robust method to follow CO_2 formation in cheese body opens wide fields of research: i) investigations on the propionic acid formation during cheese manufacturing and especially during ripening, leading to a better control of this crucial period; ii) comparisons of potential formation of CO_2 by different strains of propionic starters in true cheese environment; and iii) examination of the CO_2 content of the cheese body after cutting of the loaf and before wrapping, to better control the CO_2 content of the gas added in the packaging.

This point would allow to subsequently diminish the bringing back of cheese parts to the factories, mainly due to problems of appearance of wrapped cheese (blowing by CO_2 release in the plastic bag or sticking of the plastic film on the cheese portion, leading to a wet appearance). This point was recently examined in Canada by Fedio *et al* (1994).

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