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CONTROL AND ANALYSES OF THE
PRESERVATION OF PACKED FOODS

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HEADSPACE ANALYSIS OF PREPACKED FOODS:

a review of present techniques and development a new gas chromatograph with termal conductivity detector

- INTRODUCTION
- AN REVIEW OF THE AVAILABLE METHODS OF ANALYSIS AND ANALYSERS
- DEVELOPMENT OF A NEW ANALYSER
  - . Operating principle
  - . Instrumentation
  - . Operating procedure
  - . Experimental results
  - . Typical applications
- CONCLUSION
- SUMMARY
- BIBLIOGRAPHY

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INTRODUCTION

The conservation of many prepacked foods depends on the présence in the headspace of gases like oxygen (0_2), carbon dioxide (CO_2), amonia (NH_3), hydrogen (H_2) and nitrogen (N_2). Therefore, inert gases are often used to improve resistance to oxidation (1-11). The presence of **Oxygen** can lead to oxidation and photo-oxidation of fats, vitamins, sulphur containing amino-acids. As a result, many different undesirable substances like peroxides, adehydes, ketones and oxycholesterol (12-22) can be produced. Moreover, it is an indispensable element for the growth of yeasts and moulds. Most of the oxygen induced reactions reduce the nutritional characteristics of foods, and change their flavour or even the colour (23). The concentration of **carbon dioxide** in the headspace, can be considered as an indicator of degradation of foods by yeasts, moulds or aerobic bacteria. However, its acidic chemical and physical characteristics can also behave as a protection or simply displace the oxygen or other gases (9-24). The presence of **hydrogen** can result from the action of coliform or butyric bacteria, or it can be evidence for corrosion of the metallic can (25-26). Finally, the chemically and biochemically inert gaz **Nitrogen**, is often used to control the atmosphere around the product to be stored (27). These few examples show the importance of having simple, fast, quantitative and specific analytical methods , that can be used to analyse the atmosphère surrounding the prepacked foods.

A REVIEW OF THE AVAILABLE ANALYTICAL METHODS OF ANALYSIS AND ANALYSERS

Several methods of headspace analysis of food packages are presently used. Some analytical procedures are very specific for a certain component of the headspace. On the contrary, other methods are expecially useful for their non-specificity, and can be used to determine several components by a single measurement.

The specific measurement of **Oxygen** is done by using the following methods (28-30) :

- the particularly high paramagnetism of this molecule (31-32), can be measured by using the following analysers:(a) HELOS KV and KVS (HELANTEC AG - 4106 Therwil, Switzerland), (b) MODELS 570A, 574 and 1400/1451 SERVOMEX LTD (Crowborough, Sussex TN6 3DU,UK), (c) Oxymeters EC 18 G,EC 180, HM 18 N (HERMANN MORITZ , CHASSANT -28480 France)
- the polarographic measurement of oxygen diffusing through the membrane of the so called Clark's electrode (33), a technique applied for instance by the following analysers: (a) OXY 211 (ANATOP SCIENTIFIC INSTRUMENTS-ANATOP SA, Hünenberg 6331 SWITZERLAND) or TECAN U.S. LTD, (North Carolina 27514 USA), (b) Inpack 507 (INGOLD ELECTRODES, INC Wilmington, MA 01887, USA), (c) 340FBS (TELEDYNE ANALYTICAL INSTRUMENTS City of Industry, CA 91749 USA);
- the measurement of Nernst's potential generated by a solid electrolyte type electrode containing zirconium and yttrium oxides at high temperature (approx. 700°C). This principle is used by the analyser LC-700F by TORAY (P. Lippke GmbH & Co. D-5450 Neuwied 1) and also by the Head Space Oxygen Analyser ZR 891 - SYSTECH INSTRUMENTS Ltd (Thame - Oxfordshire- OX9 3 BX, England).

Many different methods of analysis of **carbon dioxide** have been proposed.The most promising one is probably the non-dispersive (absorption) photometry in the infrared (34). This physical principle has been applied by high performance specific analysers, usually dedicated to typical "on-line" measurements, of re-circulating gas flows . The only analysers of this type which operate without gas circulation, thus requiring only a few ml of gas sample are probably both models PA 404 SVS and 1400/1451by SERVOMEX LTD (Crowborough, Sussex TN6 3DU, England). Moreover,these instruments enable the simultaneous analysis of both oxygen and carbon dioxide.

The same principle has been applied to the "one-line" specific determination of **Ammonia**. This gas is relatively uncommon in the headspace of food products and is rarely analysed.

As far as **hydrogen** is concerned, specific sensors are also available, and they are mainly used as "leak sensors" for the security of installations, but they cannot be used without any modifications for the analyses of headspace of food products.

Finally, a commercially available instrument for the specific determination of **Nitrogen** does not exist. However, for ammonia, hydrogen and nitrogen (NH_3, H_2 and N_2), only gas chromatography with thermal conductivity detection (TCD) (35-36) can be taken into consideration. Otherwise mass-spectrometric methods by using (a) Quadruvac PGA 100 (EYBOLF-HERAEUS GmbH. - 5000 West Germany) or (b) spectromass (Congleton, Cheshire CW 12 4XR, UK) or (c) Dycor M/MA Quadrupole Analyser (AMETEK,Thermox Instrument Division - Pittsburg, PA 15238).

Analysers of this type cannot be found "turnkey" on the market : only basic equipment (GC, valves, columns etc...) that have to be modified and adapted to the specific application can be found (37-44). The present study illustrates an analyser designed and assembled for the headspace analysis of prepacked diary products, such as milk powder milk, condensed milk, cheese in can or packed with plastic films, as well as yoghurts.

DEVELOPMENT OF A NEW ANALYSER

- Operating principle

The described system is based on the gas-solid separation of the components of a gaseous mixture (H_2, N_2, O_2, CO_2 or/and NH_3) (45) using a modified gas chromatograph (Perkin-Elmer model Sigma 300), equiped with three columns and a thermal conductivity detector (fig. 1 to 4).

This type of configuration, called "Gas Sampling with Column Sequence Reversal", includes an automatic 10 ports valve connected to two chromosorb 103 columns (C_1 and C_3), mounted in series that can separate the polar gases (CO_2 and NH_3). In addition there is a molecular sieve column (C_2) for the separation of permanent gases (H_2, 0_2, N_2). The ten ports valve is driven by a pneumatic system that switches between the two positions : - (Pos.B: C_1 -> C_2 -> C_3 <=> Pos. A: C_2 -> C_1 -> C_3) . The sample to be analysed is introduced by means of a sample loop. (47).

This variable configuration avoids the problems derived from the practically irreversible adsorption of the polar gases on the molecular sieves. Therefore, the permanent gases flow twice through the chromosorb C_1 column: the first time (Pos.B) they are not separated (air), the second time (Pos.A), they flow already separated by the molecular sieve column as hydrogen, oxygen and nitrogen.

The presence of a third column is not common: this modification has been done in order to improve the separation of the positive peak of carbon dioxide , from the negative peak resulting from a pressure instability during the switching of the ten-ports valve (see fig. 5A).The molecular sieve column cannot separate argon from oxygen. This limitation can be overcome by considering that the ratio Ar/N_2 is constant (=0.0119). In fact, in most cases, it is possible to evaluate the argon concentration in an unknown mixture on the basis of the measured nitrogen concentration.

Helium is generally used as a carrier gas, (48). Some limitations can be found for a precise and accurate determination of the concentration of hydrogen. As pure hydrogen presents a greater thermal conductivity than helium, it should theoretically produce a negative peak. In fact, the polarity of the signal depends on hydrogen concentration. At a concentration of approximately 10% of hydrogen in helium, the signal is positive.

Madison (49) refers this behaviour to the existence of a minimum point of the thermal conductivity of the H_2/He mixtures. In relationship with the different concentrations, negative or even double peaks can be observed. In order to avoid difficulties of this kind, it is advisable to use argon as carrier gas whenever is important to establish the hydrogen concentration.

The described system allows the determination of the relative composition (% vol/vol) of the gases if the sample is injected using a gas syringe (the pressure in the syringe is generally unknown). It also allows the determination of the absolute composition, if the sampling is done directly into the headspace of a rigid package (for instance a metal can), using an adequate sample collecting device. In this case, the determination of the pressure and the "true" volume of the headspace of the package are both important and necessary.

The determination of certain constituents, like carbon dioxide and ammonia , can markedly depend on the environment of the gaseous sample (pH, water content, ionic strength and composition of the matrix). These parameters have to be taken into account when results are evaluated.

- Instrumentation

The analyser is constituted by the following parts (in alphabetical order):

AS: sampling loop of 0.1 or 0.5 or 1.0 or 5.0 ml capacity (Perkin-Elmer item number 0332-4901 or 0332-4902 or 0332-4903 or 0332-4904 respectively)

C_1: column filled with chromosorb 103 (Supelco part number 2-0217; 80 / 100 mesh; 1.25m; stainless steel 1/8")

C_2: column filled with molecular sieves 5A (Supelco part number 2-0301; 45 / 60 mesh; 4.50m; stainless steel 1/8")

C_3: column for both improved separation and additional retention or delay,filled with chromosorb 103 (Supelco part number 2-0217; 80 / 100 mesh; 1.80 m;stainless steel 1/8")

C_4: uncoated capillary column to limit backflow (typically 10 cm; fused silica or glass 0.21 mm ID)

DE: vacuum system equipped with a vacuum pump (AEG AD71N24)

DP: sampling device for metallic cans (Nestec SA, part number 853000, 1800 Vevey, Switzerland) or equivalent

FD: exponential dilution flask (Varian, part number 96-000038-00) (36)

HWD or TCD: glass-coated filament type, thermal conductivity detector (Perkin-Elmer, part number 0331-AC00). Bridge current adjusted to 65 mA for Ar and to 240 mA for He

IE: electronic integrator (Perkin-Elmer LCI 100)

MN: pressure gauge (0-2000 mbar) with digital display (Leybold-Heraeus: PIEZOVAC pressure gauge,part number PV 111 with measurement cell EDA 420-311.215 - A 15)

R_1 to R_4: two-port valves (Supelco part number 2-2139)

R_5, R_6: three-port valves (Varian, part number 88-730900-00)

S_1: gas syringe:

- 10 ml capacity (1010TTL Supelco, part number 2-1000) with valve and side opening needle (part number 2-1743) for the collection of samples in flexible packages
- 500 ml capacity (Varian,part number 89-988965-00) for calibration

S_2: gas-tight syringe for the injection for a volume V2 of gas into the system (for example 25 ml;1025TTL Supelco,part number 2-0683)

TF: flexible hose (Approx. 2 cm) for connection to syringe S1 or to dilution flask FD

VC: ten-ports valve with pre-programmed pneumatic switching (Valco part number A60),for use at ambient temperature

Calibrating gas: N_2, Carbagas, grade 52, class I
O_2, Carbagas, grade 48, class I
H_2, Carbagas, grade 57, class I
CO_2, Carbagas, grade 48, class I
NH_3, Carbagas, grade 45, class I
as well as different calibration mixtures of these pure gases, as desired.

Operating procedure

- Loading the sampling loop with a gas syringe (Fig. 1):

After having sticked a self-adhesive septum onto the package (Gummi Maag, 3172 Niederwangen, Switzerland, part number 128078), the sample is collected using sampling syringe S_1 which has previously been purged using the carrier gas. This syringe, which contains a volume V_1 of gas to be analysed, sufficient to purge the circuit indicated in thick lines in fig.1, is connected to the valve R_1 by the flexible hose TF.The switching valve VC is in position A. The capillary tubing C_4 serves as a vent, which brings the sampling loop AS to atmospheric pressure, thus avoiding practically all air blackflush. This operation produces a momentary over-pressure. The sample in the sampling loop AS can be injected into the GC as soon as the pressure MN indicates atmospheric pressure again (acceptable difference is approx. 10 mbar).

- Loading the sampling loop from the metallic can (Fig.2)

First of all, the complete circuit indicated in thick lines in fig. 2, has to be evacuated by using the vacuum system DE (residual acceptable pressure is 5 to 10 mbar approx.).

The switching valve VC is in position A. The sampling device DP must be in tight contact with the cover of the can. (*)

Once the vacuum is established, the valve R_3 is closed in order to monitor the sealing of the system. The lid of the can is pierced using the device DP. The pressure is measured when it settles again. Its value can be, theoretically, above, equal or lower than the atmospheric pressure, depending on the pressure found inside the can to be analysed. The valves R_5 and R_6 are commuted so that they are connected, but leaving the sampling loop isolated (see fig.3). The loop is filled and ready for injection into the gas-chromatographic system.

- Chromatographic separation (Fig. 3 and 4):

After the manual loading of the sampling loop AS, the analysis is entirely driven by the microprocessor of the GC. First of all, the valve VC is switched on to the position B (= time t_1 see fig.3). The carrier gas, then, carries the sample through the column C_1, where the polar gases are separated (CO_2 or NH_3). The permanent gases (H_2, O_2, N_2) run very quickly through the column without being separated. As soon as the permanent gases reach the column C_2 (= time t_2),the valve VC is pneumatically switched back to its initial position A (fig. 4), which avoids trapping the polar gases in a practically irreversible manner on the molecular sieves contained in the column C_2.

(*) A few drops of glycerine deposited on the lower surface of the rubber plug will improve the adherence of the piercing device onto the lid of the can.

The ideal moment t_2 for switching is empirically determined by the injection of the air as a sample. t_2 has to be increased gradually up to the disappearence of the "air" peak which must be entirely resolved into the peaks of nitrogen and oxygen. An additional increase of t_2 can lead to a systematic error,deriving from the elimination of some peaks of the polar gases, especially the carbon dioxide.

Separation is performed under the following conditions:

- Injector loop temperature: 150°C
- Oven temperature (with columns C_1, C_2 and C_3): isothermal at 95°C
- Detector: Catharometer or thermal conductivity type (HWD): 200°C
- Switching time: t_1 = 0.01 min; t_2 = 0.90 min
- Carrier gas flow through the columns: 20 ml/min (He or Ar)
- Carrier gas back-pressure at GC inlet: 380 kPa

Fig. 5 shows an example of the chromatographic separation obtained under the conditions mentioned above (CO_2 and NH_3 injected separately).

The optimisation of the chromatographic conditions can be done by trial and error, on the basis of the separation of the peak of ammonia, which is very temperature dependent . This peak must be located between carbon dioxide and hydrogen. All peaks of the polar gases present a certain "tailing" effect, especially remarkable at low temperature for ammonia and at high temperature for carbon dioxide.

- Determination of volume Vx and pressure Px in the headspace of the can (fig.4):

During or after chromatographic separation, it is possible to determine the "real" volume Vx in the headspace of the can being analysed.

This headspace includes the apparant and intersticial gaseous volumes in the powders and other solid materials contained in the can. For this purpose, the valve R_4 is opened and the volume V_2 is introduced into the circuit indicated in thick lines in fig.4 (can included) using the syringe S_2. This volume could be constituted by air for example.The pressure increases, from P_1 to P_2 (read after equilibration). The volume of gas to be injected must be at least in the order of 20 to 30% of the volume Vx to be determined. By applying Boyle-Mariotte's law twice, both Vx, and Px can be successively calculated, where Py is the original pressure in the can before piercing. Calculations are shown below. During this procedure, the system must be completely sealed,sampling device PE included (*).

- Calibration

If the size of the sample is large enough, it is useful to start with a preliminary analysis, just before a more accurate calibration of the analyser. Otherwise, it will be sufficient to use the regression coefficients (Table 1) of the calibration curves (fig. 6, A and B) calculated for each individually gas following the method of the external standard.

Up to concentrations of about 10% vol/vol, the calibration curve is established by using a dilution flask (36), which works on the principle of the exponential dilution.

$$C_t = C_o \; e^{-Qt/V}$$

where: C_t = concentration at time t

C_0 = concentration at time zero (injection of gas into dilution flask)

Q = flow of carrier gas through the dilution flask

V = volume of dilution flask

(*) A few drops of glycerin deposited on the lower surface of the rubber plug will improve the adherence of the piercing device onto the lid of the can.

The dilution flask FD is directly connected to the flexible hose TF (fig.1) instead of to the syringe S1.

More adequate calibration techniques have been proposed (50-51) for concentrations higher than 10 or 20%.

For this study, we have used a simple technique consisting in collecting successively known volumes of different pure calibrating gases, where the pressure has been reduced to atmospheric through an opening T (vent).By simple diffusion into the syringe, gas can be mixed in any proportion. This syringe allows loading of the sampling loop AS (S_1 in fig.1).

- Calculation procedure

The **relative concentrations** (% vol/vol) are calculated using the 100% peak normalisation technique by applying regression coefficients as in Table 1.

The **absolute concentration** (mmol/l) is calculated after having previously determined the real volume Vx and pressure Px of the headspace of the package. For this purpose, Boyle-Mariotte's law is applied several times. We assume that this is valid for all analysed gases:

Determination of V:

$$P_1 (V_{pc} + V) + P_{atm} (V_2 + V_{junct}) = P_2 (V_{junct} + V_{pc} + V_x)$$

(before the injection of V_2) (after the injection of V_2)

Where:

V_x = real volume of headspace to be determined

V_2 = volume of gas (air) injected into V_{pc} and V_x using syringe S_2

V_{junct} = dead volume of the connection S_2-R_4, determined by filling it with water and weighting

V_{pc} = volume of the small circuit indicated in thick lines in fig.4, delimited by R_4, R_3, R_2, MN, R_5, R_6 and DP (see below)

P_1 = pressure read on digital pressure gage MN before injection of V_2

P_2 = pressure read on the digital pressure gauge MN after the injection of V

P_{atm} = atmospheric pressure

Determination of P_x:

$$P_x (V_x) + P_o (V_{gc}) = P_1 (V_{gc} + V_x)$$

(before piercing) (after piercing)

Where:

P_x = internal pressure of the can before it is pierced

P_o = residual pressure after evacuating the large circuit by using the vacuum system PE. P_o is considered as negligeable in this calculation if the vacuum is sufficient (tolerance: 5 to 10 mbar)

V_{gc} = volume of the circuit indicated in thick lines in fig 1, which corresponds to the small circuit V_{pc} of fig. 4 and additionally the loop located between R_5 and R_6, passing through the sampling loop AS (see below)

Determination of the total amount of gas "n" :

Px and Vx having been calculated, it is possible to calculate the total amount of gas "n" (in moles) contained in the head space of the can, by using the law of gases. Then, the quantity of each component in the mixture and its relative concentration can be determined.

A more accurate calculation can be accomplished by taking into account the partial molecular volumes of each gas in the mixture.

Determination of V_{pc} and V_{gc}:

The volumes of the small circuit V_{pc} and the large circuit V_{gc} are definitely determined by using again Boyle-Mariotte's law:

For the small circuit: $P_{atm} (V_2 + V_{junct} + V_{pc}) = P_3 (V_{junct} + V_{pc})$

For the large circuit: $P_{atm} (V_2 + V_{junct} + V_{gc}) = P_4 (V_{junct} + V_{gc})$

where P_3 and P_4 are pressures read on the pressure gauge after injecting volume V_2 into the small circuit (fig. 4) and respectively, into the large circuit (fig. 1). These determinations do not require a can, but must performed with accuracy. In fact, both volumes V_{pc} and V_{gc} appear in the course of all calculations for V_x and P_x. At least ten measurements should be preferably done. V_{gc} depends on the size of the sampling loop being used.

Experimental results

Calibration

Figures 6 (A and B) show calibration curves obtained separately for carbon dioxide, ammonia, hydrogen, nitrogen and oxygen in helium (fig.6A) and in argon (fig.6B) as carrier gases. Calibration mixtures are prepared using a syringe (S1) of 500 ml capacity, according to the technique recommended for high concentration (>10%: fig.1).

Tables 1and 2 indicate the linear regression coefficients (α, β) and quadratic (a, b, c) coefficients calculated for the calibration curves. The coefficient of the quadratic term (c) could be neglected for certain gases (N_2 and O_2 in Ar, or possibly in He).

This would eventually allow the use of the first order coefficient b only, or its reciprocal 1/b, the response factor of the detector. An approximation of this nature is more accurate whenever is possible to interpolate within a more restricted range defined by two consecutive points.

This is the reason why it is useful to perform a preliminary tentative analysis, if enough volume of the headspace is available for a few analysis. Nevertheless,it is generally preferable to employ more precise quadratic regressions by using modern calculators.

Figures 7 (A, B) show the calibration curves obtained with helium as carrier (fig.7A) and argon (fig. 7B) . The exponential dilution flask FD was used according to the technique recommended for low concentration levels (less than10% : fig. 1).

Tables 3 and 4 indicate the corresponding linear and quadratic coefficients. Except for the calibration curve of oxygen in helium as carrier gas, it can be verified that the simple linear regression is not sufficient to evaluate the results of the analyses.

Detection limits:

Detection limits can be established by injecting decreasing concentrations of single pure gas into the carrier gas. They correspond approximately to a signal/background noise ratio of 10 (Table 5). The most restrictive factor for the detection limit, as well as for the repeatability of the measurements, is the contamination by ambient air, especially during sampling.

Repeatability

The repeatability has been calculated on the basis of triplicate determinations (Fig. 6 and 7). Best values are obtained for high concentration levels : the coefficient of variability ranges from 0.1 and 1.0% relative, for concentrations above 40% vol/vol.

For concentration below 40% the coefficient of variability ranges from 1 to 4% relative. The best repeatability has been obtained using pure calibration gases (100% vol/vol). This indicates that the main source of errors is the preparation of the calibrating mixture, not the measurement itself.

Typical applications:

A first example of application consists in the analysis of the composition of the headspace of yoghurts during storage for 3 weeks at 7°C. The application of this new technique to the measurement of the partial pressure of oxygen pO_2 in viscous product of this type (52) generates some interesting considerations:

- does yoghourt consume oxygen and does it produce carbon dioxide in the course of this type of storage?

- is the mass of yoghourt in a state of equilibrium with its headspace?

In order to answer these questions, the yoghourt samples have been stored under the previously mentioned conditions and the headspace measured before and after having vigorously shaken identical pots (Table 6).

Due to the fact that a diffusion of the gases through the packaging material (constituted by a sheet of polystyrene) is possible , this sheet was covered by a bonded aluminium sheet,in order to create an air-tight complex.

The conclusion that can be drawn from these experiments are:
- the yoghourts continue to consume oxygen of the headspace even at this temperature, but do not produce carbon dioxide as long as no yeast or moulds appear on the surface;
- due to the very slow diffusion of the gases from or into the mass of the yogourt, this does not reach a state of equilibrium with its headspace.

The second example of application concerns the analysis of gases present in the headspace in a can made of tin. The appearence of this container was suspect because it was markedly blown out, and contained 1kg of melted cheese .The following concentrations (% vol/vol) have been found:

CO_2: 22.9 NH_3: absent H_2: 63.6 N_2 : 11.1 O_2 : 2.4

The ratio O_2/N_2 = 0.216 < 0.268 (in air) indicates a slight consumption of oxygen in the course of the alteration of the product. One can observe a remarkable production of carbon dioxide and hydrogen, both deriving from butyric bacteria degradation. Their presence has been confirmed later by a GC analysis of the content of butyric acid in the cheese itself.

CONCLUSIONS

The use of commercial sensors mentioned at the beginning of this study is certainly a very convenient solution for the determination of a single gas, for instance O_2. Analysers of this type are in fact: specific of fast response, relatively inexpensive, compact and even portable. They can thus be used directly in the production plant itself. They are designed for general routine controls (on-line quality control). The ease of use does not require specialized personnel.

The analyser proposed in this study, does not substitute the mentioned sensors, but only constitutes a complementary technique. In fact, it enables the simultaneous determination of several gases in the same sample. Moreover, it is dedicated to more complex analyses and to research and development programs.

For most dairy products, the simultaneous determination of several gases (O_2, CO_2, H_2 and N_2, possibly NH_3) can produce

interesting data about possible degradations and the probable cause (coliform, propionic, butyric bacteria, corrosion in the can) (9, 25, 26). It allows verifying the origin and the development of the microbial flora (bacteria, yeasts, moulds). For this purpose, the determination of nitrogen allows calculating both the balances (O_2 + CO_2 + H_2 + N_2) and the ratios (O_2/N_2,CO_2/N_2, H_2/N_2) of the individual components, in which nitrogen is considered as a non-variable element (" internal standard").

The analyser recently proposed by Bertoli et al. (53) is designed to take into account the deformations that metallic cans may present if they are partially filled under vacuum (550 to 850 mbar).This is usually not necessary with dairy products, which are generally packaged under controlled atmosphere (CO_2, N_2) at a pressure close to atmospheric .

The proposed analyser can also be used for many other applications, like the determination of gas composition into the eyes of cheese, or into large enclosures (cheese cellars, bioreactors and fermenters) . The selection of the adequate sampling syringe for each specific situation is important.

SUMMARY

After having briefly mentioned the positive and negative effects deriving from the presence of certain gases (O_2, CO_2, NH_3 and N_2) in the headspace of some prepackaged food products, this study provides an overview of the principles of measurement of these gases, and the related commercially available analysers. These sensors are designed for applications in large batch analysis with advantages deriving from their high rates of specificity and rapid measurement, reasonable price, limited usage of space, but they can only dose one single component at a time in the gas mix, two at the utmost.

This study proposes a new analyser that provides the possibility of analysing simultaneously all above mentioned gases.It is based on a gas/solid chromatographic separation with catharometric sensing TCD).The adopted configuration is particularly dedicated to the analysis of dairy products such as powder milk and canned condensed milk, cheese in can or packaged under plastic film, and yoghourt in pots. After explaining the operating principle of the analyser, the study describes the design, the use, the optimisation of the operating parameters, the calculation of the results and the calibration techniques. Detection limits and repeatability of the measurements are documented. The analyser can be used for many other applications, like the determination of the gas composition in the eyes of cheese , in the cellars, bioreactors and fermenters, provided an adequate sampling syringe is used in each situation.

Acknowledgments

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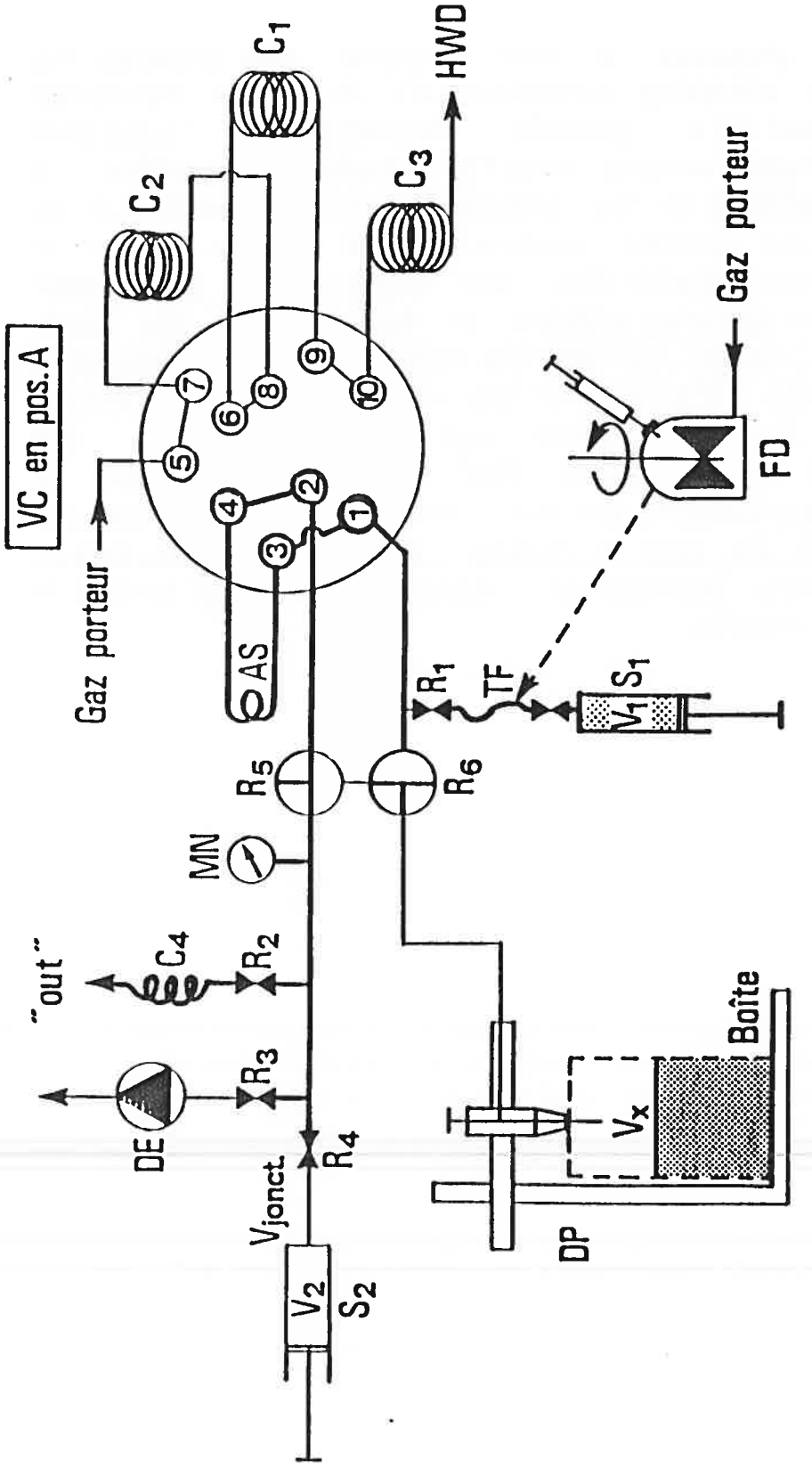

Fig. 1: Configuration adopted to load the sampling loop AS by using the sampling or calibrating syringe S_1 or the exponential dilution flask FD

note: Switching valve VC: in position A
Pressure read on pressure gauge MN:Patm(atmospheric)

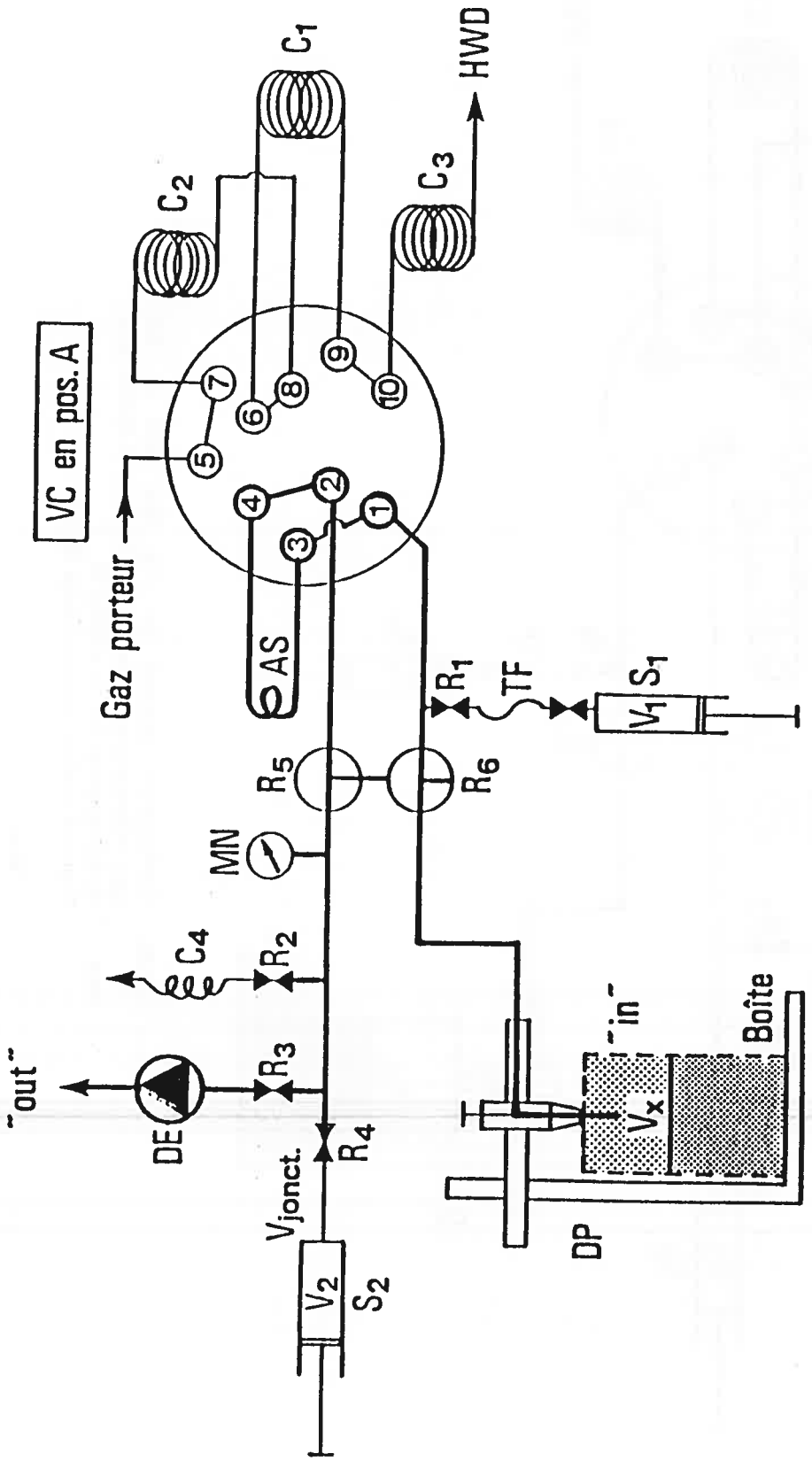

Fig. 2: Configuration adopted to load the sampling loop AS by using the perforation and sampling device DP for metallic cans

note: Switching valve VC: in position A
Pressure read on gauge MN: P_1, (after perforation)
The circuit indicated in thick lines is the large circuit (see text)

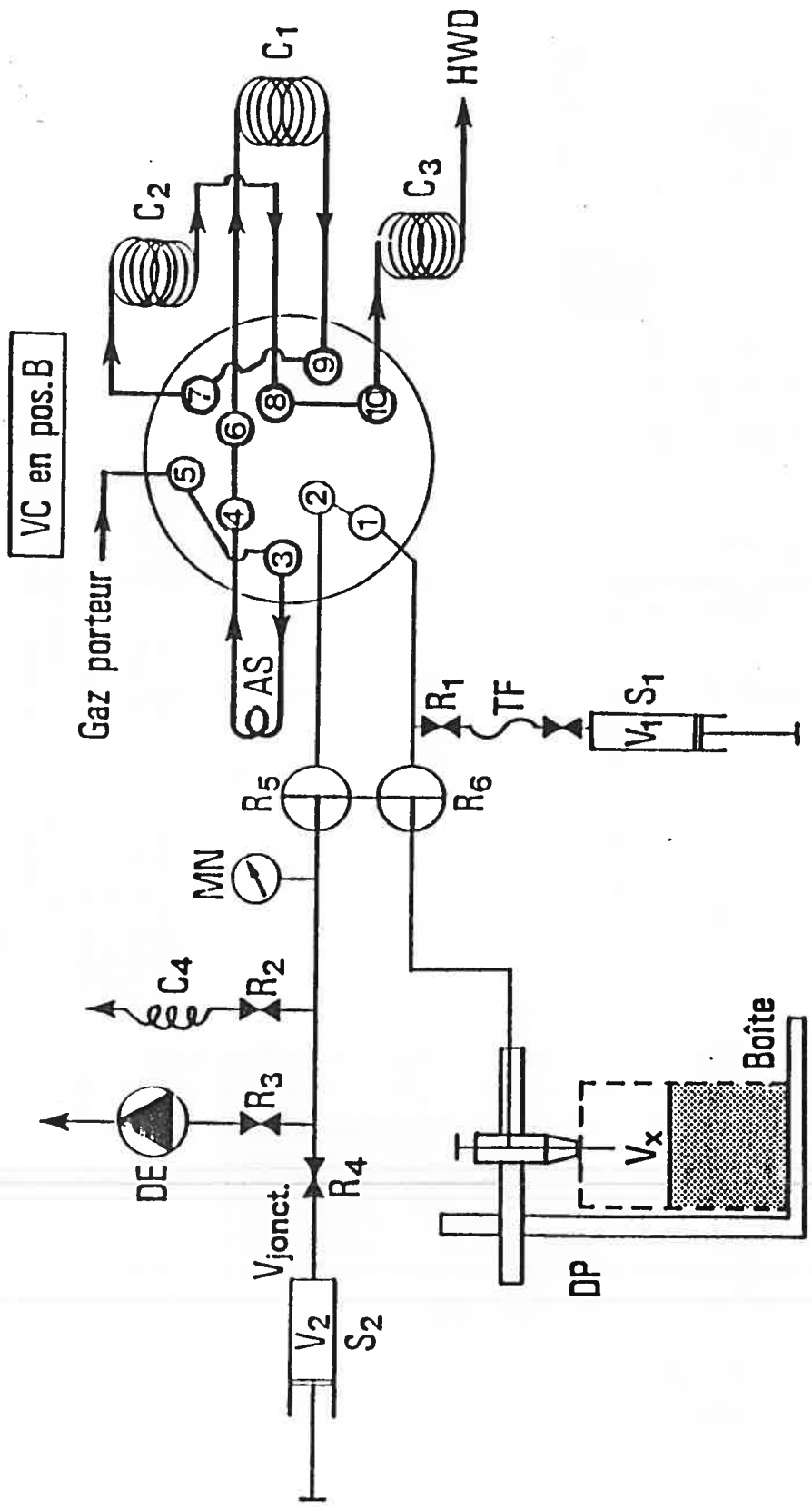

Fig.3: Configuration adopted for the first part of the gas chromatographic separation (from t_1 to t_2,see text)

note: Switching valve VC: in position B
Pressure read on pressure gauge MN: P_1

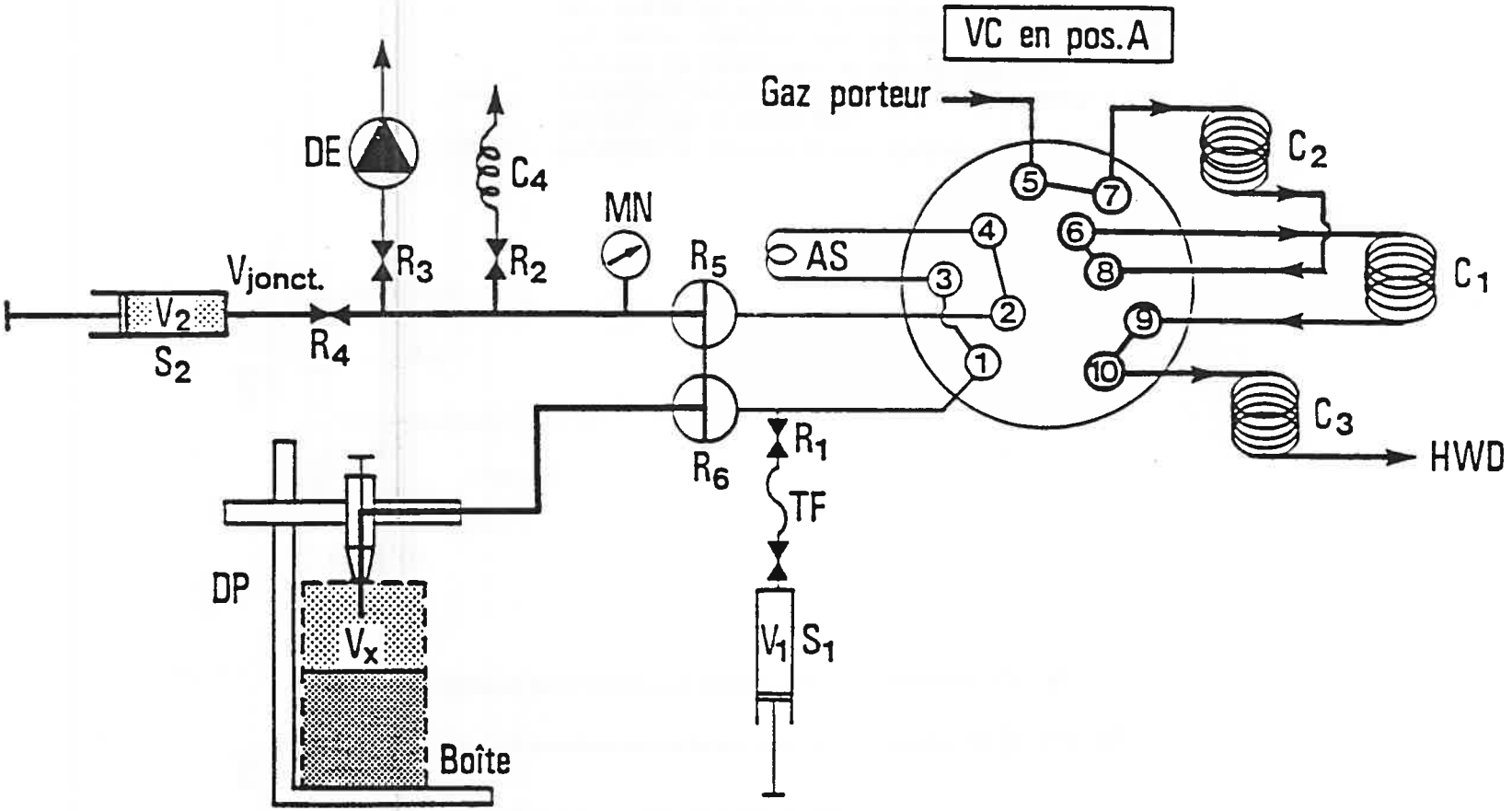

Fig. 4: Right-hand side of the figure:
Configuration adopted for the second part of the gas-chromatographic separation (after t_2, see text)
Left-hand part of the figure:
Configuration used to determine the real volume Vx in the headspace inside a metallic can

note: Switching valve VC: in position A
Pressure read on pressure gauge MN: increases from P_1 to P_2 (injection V_2)
The circuit indicated in thick lines is the small circuit (see text)

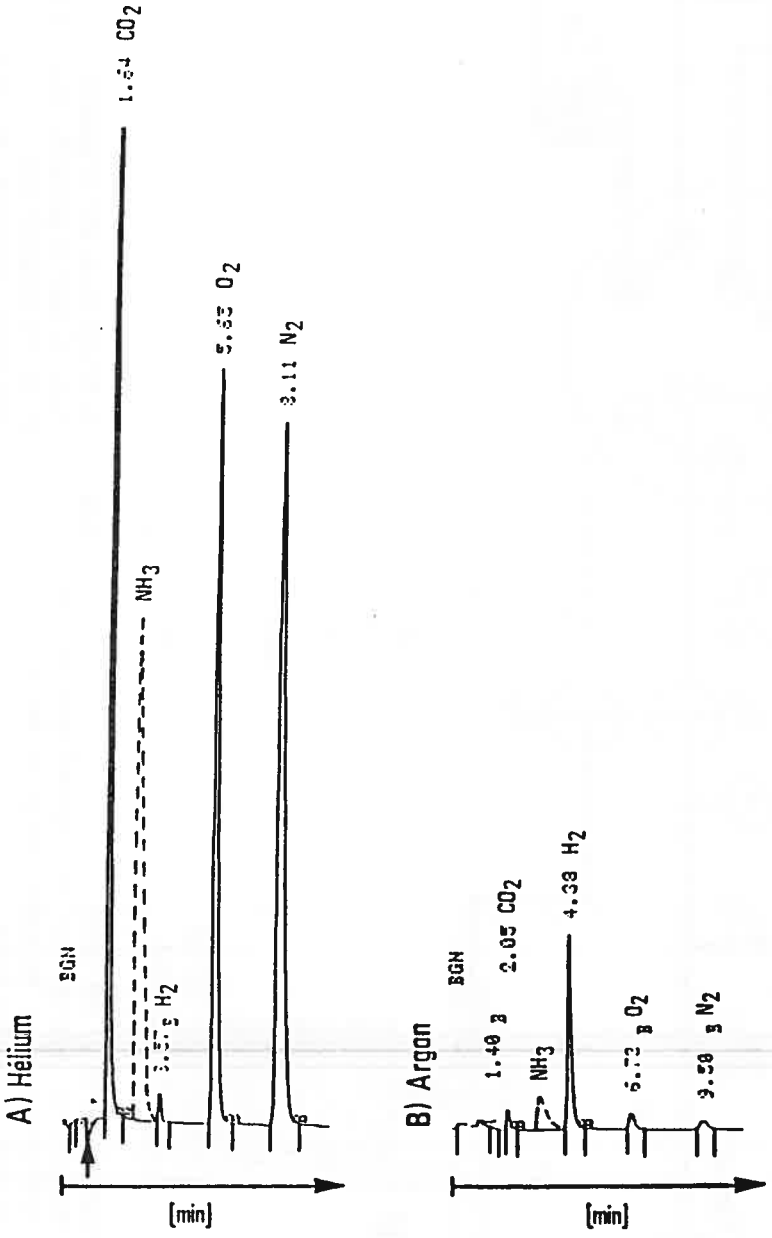

Fig.5: Examples of chromatograms obtained with helium (A) and argon (B) as carrier gas.

note: Composition of the gas mixture: 20% vol/vol each
Ammonia (in dotted line) is injected separately
The arrow indicates the position of the negative peak due to the change of pressure during injection (t_1)

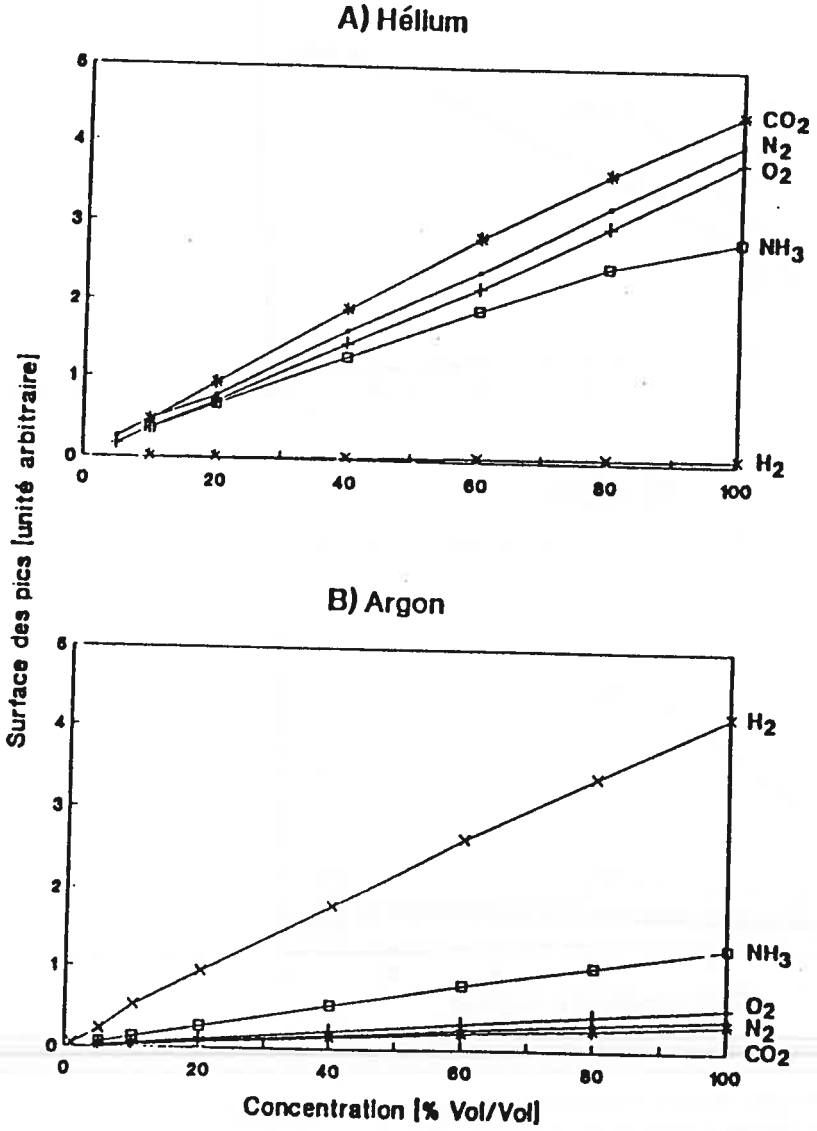

Fig. 6: Calibration curves obtained by using helium (A) and argon (B) as carrier gas, and sampling with the calibrating syringe

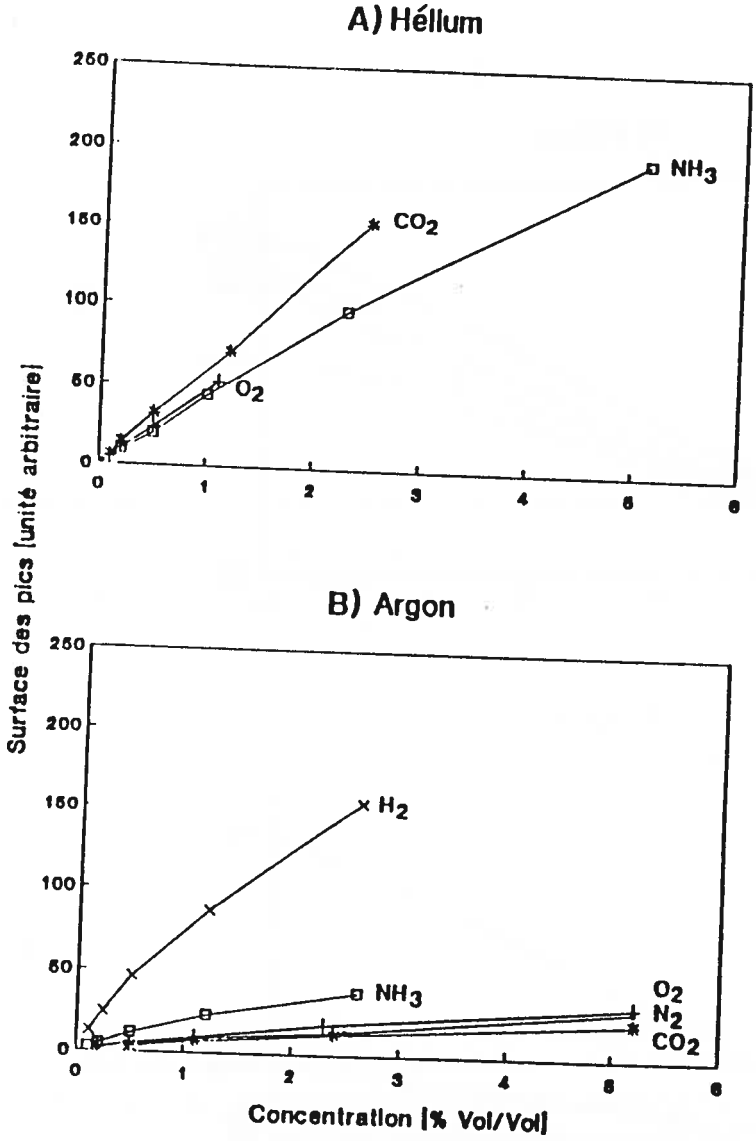

Fig. 7: Calibration curves obtained by using helium (A) and argon (B) as carrier gas, sampling with the exponential dilution flask

Table 1: linear[1] (α, ß) and quadratic[2] (a, b, c) regression coefficients as calculated from the calibration curves obtained using helium as carrier, by injecting with the calibration syringe S1 (Fig.7A)

Gas	HELIUM				
	α	ß	a	b	c
CO_2	82'200	44'100	-80'000	53'300	-84,7
NH_3	135'000	28'100	-28'900	37'300	-85,4
H_2	8'000	565	3'430	824	-2,38
N_2	34'900	39'900	58'900	38'100	17,1
O_2	-38'900	37'900	6'500	34'600	32,5

These coefficients have been calculated for the following types of regressions:

1) linear: $y = \alpha + \beta x$ et
2) quadratic: $y = a + bx + cx^2$
   where y is the area of the peak as determined by GC (arbitrary units), and x is the relative concentration of the component (as % vol/vol)

Table 2: Linear [1] (α,β) and quadratic[2] (a,b,c) regression coefficients calculated from the calibration curves obtained using argon as carrier, by injection with the calibration syringe S1 (Fig. 7B)

Gas	ARGON				
	α	ß	a	b	c
CO_2	1'450	3'320	-2'650	3'610	-2.93
NH_3	7'560	12'800	-2'680	13'600	- 7.32
H_2	69'500	41'600	10'300	47'200	- 57,5
N_2	3'860	4'000	1'420	4'190	- 1,74
O_2	2'510	5'340	-24,5	5'520	-1,81

These coefficients have been calculated for the following régression types:

1) linear: $y = \alpha + \beta x$ and
2) quadratic: $y = a + bx + cx^2$
   where y is the area of the peak as determined by GC (atbitrary units), and x is the relative concentration of the component (as % vol/vol)

Table 3: linear [1] (α, ß) and quadratic[2] (a, b, c) regression coefficients calculated from the calibration curves obtained using helium as carrier, by injecting with the exponential dilution flask (Fig. 8A)

Gas	HELIUM				
	α	ß	a	b	c
CO_2	503	60'500	-1'450	57'200	1'250
NH_3	3'990	38'000	-859	45'900	-1'460
H_2	n.d.[3]	n.d.[3]	n.d.[3]	n.d.[3]	n.d.[3]
N_2	-419	67'000	-1'820	86'400	-34'900
O_2	258	47'50	230	47'700	-136

These coefficients have been calculated for the following regression types:

1) linear $y = \alpha + \beta x$ et
2) quadratic $y = a + bx + cx^2$
   where y is the area of the peak determined by GC (arbitraty units), and x is the relative concentration of the component (as % vol/vol)
3) not determined

Table 4:linear[1](α, ß) et quadratic[2] (a, b, c) regression coefficients calculated from the calibration curves obtained using argon as carrier, by injecting with the exponential dilution flask (fig.8B)

Gas	ARGON				
	α	ß	a	b	c
CO_2	1'870	3'970	717	5'800	-332
NH_3	3'390	13'900	723	22'900	-3'310
H_2	12'700	55'700	5'590	78'600	-8'340
N_2	1'840	5'310	2'050	5'080	38,8
O_2	3'110	5'730	1'240	8'680	-535

These coefficients have been calculated for the following regression types:

1) linear $y = \alpha + \beta x$ et
2) quadratic $y = a + bx + cx^2$
   where y is the area of the peak determined by GC (arbitraty units), and x is the relative concentration of the componen (as % vol/vol)

Table 5: Détermination of détection limits obtained using the described method

Gas	detection limit (% Vol/Vol)	
	in He[1]	in Ar[2]
CO_2	0.05	0.5
NH_3	0.1	1.0
H_2	n.d.[3]	0,1
N_2	0,1	0,5
0_2	0,1	0,5

1) Détermined using a sampling loop AS of 0.1 ml

2) Détermined using a sampling loop AS of 0.5 ml

3) not determined

Table 6: Composition of the headspace of the natural yoghourts during storage at 7°C, without shaking, or after vigorous shaking just before sampling

Time of storage (days)	pre sampling treatment	Composition of the headspace of the yoghourt					
		Conc. CO_2 %vol/vol	Conc. O_2 % vol/vol	Conc. N_2 % vol/vol	Conc.tot* % vol/vol	Ratio O_2/N_2	Ratio CO_2/N_2
7	not shaken	$\bar{x}$ = 15,3 s = 0,67 cv= 4,36	$\bar{x}$ = 15,3 s = 0,15 cv= 1,00	$\bar{x}$ = 68,2 s = 0,25 cv= 0,37	$\bar{x}$ = 98,8 s = 0,71 cv= 0,72	$\bar{x}$= 0,224	$\bar{x}$= 0,224
7	shaken	$\bar{x}$ = 21,0 s = 0,92 cv= 4,36	$\bar{x}$ = 13,7 s = 0,55 cv= 4,02	$\bar{x}$ = 63,9 s = 0,26 cv= 0,41	$\bar{x}$ = 98,6 s = 0,25 cv= 0,25	$\bar{x}$= 0,214	$\bar{x}$= 0,329
14	not shaken	$\bar{x}$ = 16,1 s = 0,20 cv= 1,24	$\bar{x}$ = 13,6 s = 0,25 cv= 1,86	$\bar{x}$ = 70,1 s = 0,87 cv= 1,24	$\bar{x}$ = 99,8 s = 0,93 cv= 0,93	$\bar{x}$= 0,194	$\bar{x}$= 0,230
14	shaken	$\bar{x}$ = 21,2 s = 1,15 cv= 5,44	$\bar{x}$ = 12,2 s = 0,55 cv= 4,53	$\bar{x}$ = 66,8 s = 0,67 cv= 1,00	$\bar{x}$ = 100,2 s = 0,10 cv= 0,10	$\bar{x}$= 0,183	$\bar{x}$= 0,317
21	not shaken	$\bar{x}$ = 16,4 s = 0,83 cv= 5,08	$\bar{x}$ = 12,2 s = 0,31 cv= 2,50	$\bar{x}$ = 70,7 s = 0,44 cv= 0,62	$\bar{x}$ = 99,3 s = 0,67 cv= 0,67	$\bar{x}$= 0,173	$\bar{x}$= 0,232
21	shaken	$\bar{x}$ = 21,4 s = 0,65 cv= 3,04	$\bar{x}$ = 10,8 s = 0,40 cv= 3,74	$\bar{x}$ = 66,8 s = 0,35 cv= 0,53	$\bar{x}$ = 98,9 s = 0,25 cv= 0,25	$\bar{x}$= 0,162	$\bar{x}$= 0,320

*) Total conc. = Conc. CO_2 + Conc. O_2 + Conc. N_2 (calculated, not measured)

$\bar{x}$ = average, s = deviation and cv = coefficient of variation (as %) for triple determinations

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