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Development of an Analyser to Determine the Oxygen and Carbon Dioxide Levels in the Head-space of Packages

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This paper describes the development of an analyser intended to measure the concentrations of CO_2 and O_2 present as part of the gas mixture in the head-space of food packages. The efficiency of the measuring device was evaluated by analysing the atmosphere of packages containing the following food products: grated Parmesan cheese, milk powder and fresh pasta. A comparison between the results of the analysis performed with the newly developed analyser and those obtained by gas chromatography showed good correlation between the two methods. The O_2 and CO_2 analyser presented satisfactory accuracy (absolute error smaller than 2.5%, v/v) and precision (relative standard deviation varying from 0.2% to 8.5%). In addition, final results of the levels of both gas types present in the atmosphere submitted to analysis are obtained after about 2 min. The proposed analyser constitutes an alternative solution for the analysis of gases present in the head-space of packages, especially when gas samples of very small volumes—about 200 µl—are to be used. Copyright © 1999 John Wiley & Sons, Ltd.

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INTRODUCTION

The bacteriostatic and fungistatic effect of CO_2 present in atmosphere of food packages is well known.[1] However, the effect of O_2 largely depends on the characteristics of each individual food product, since the presence of this gas causes quick deterioration of foods, both by the growth of spoilage bacteria and chemical and biochemical breakdown processes.[2–4] In other cases, the presence of O_2 may be desirable, as in packages containing red meats, where it contributes to keeping the shiny red colour of the packed product.

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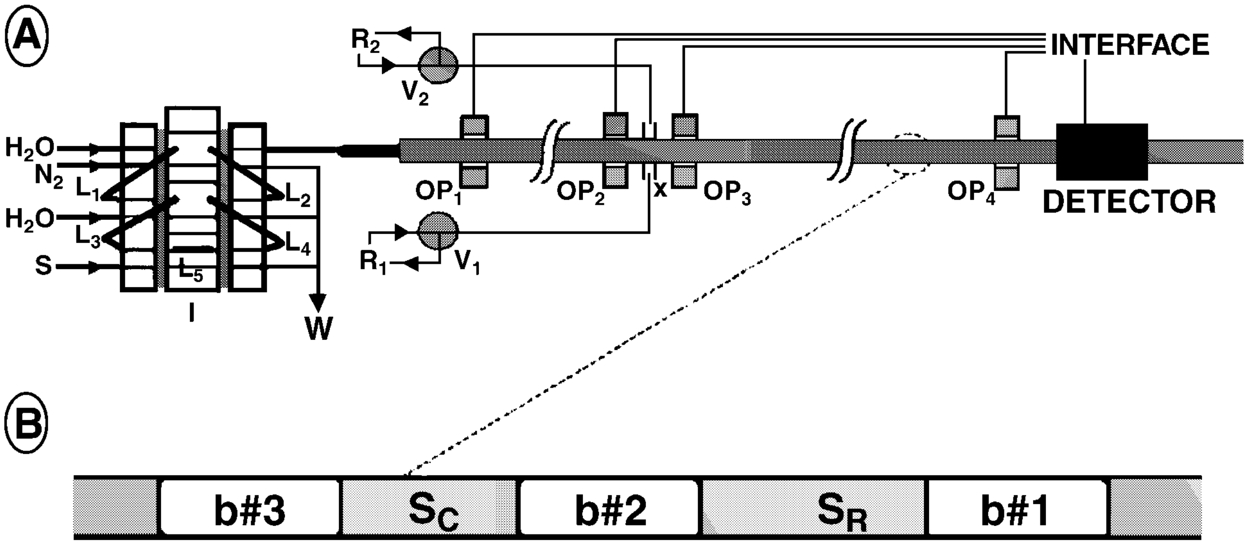

Figure 1. (A) Bisegmented flow system used for volumetric and spectrophotometric determination of gases. V_{1-2} electromechanical values; OP_{1-4}, optical switches; I, proportional injector: S, gas sample inlet; R_{1-2}, reagent solutions; C, carrier fluid (H_2O; X, point of insertion of reagents. (B) Profile of gas sample, b#1 and b#3, bubbles containing nitrogen; b#2, bubble containing sample; S_R, liquid segment containing reagent; S_C, collector liquid segment.

The determination of O_2 and CO_2 in the head-space of food packages becomes necessary because a more precise control of the gas types present in food packages may help to extend the shelf-life of the packed product. The quality of a large number of food products is adversely affected during storage as a result of numerous reactions that take place between their component substances and the gases that make up the atmosphere that surrounds those products. O_2 is one of the major factors responsible for such quality losses, as it causes the oxidation of fats, pigments, aromas and vitamins with a consequently negative impact on the sensorial characteristics and nutritional value of the foods. As a result, reducing the O_2 concentration in the atmosphere of the packages retards spoilage of a large variety of foods. CO_2 can be injected into the package—in pure form or as part of a gas mixture—to slow down microbiological spoilage processes due to its bacteriostatic and fungistatic properties.

Once the composition of the gas mixture present in the head-space of the packages is known, it becomes possible to evaluate the efficiency of packaging processes, determine the barrier properties of the packages and the stability of the packed foods, and identify the possible causes of collapsed or blown packages and other quality changes that affect the food product.

Several different methods are currently available for the determination of the O_2 and CO_2 contents in the head-space of packages, such as gas chromatography,[5–7] infra-red absorption[8–9] and volumetric methods (measuring devices of Orsat).[10] In addition to these analytical techniques, several devices specifically designed for the determination of O_2 and/or CO_2 levels in packages of foods, beverages and chemical products are commercially available today. However, these devices require high volume samples (5–30 ml). The analyser described in this paper and specifically designed to determine the O_2 and CO_2 levels in the head-space of food packages requires gas samples the volume of which varies from about 100 μl to 200 μl.

MATERIALS AND METHODS

The analyser developed in a previous stage of the research project was used to determine the O_2 and CO_2 concentrations present in modified atmosphere packages of three different products: milk powder, grated Parmesan cheese and fresh pasta. The same packages were submitted to analysis by gas chromatography to verify the accuracy of the proposed analyser.

Reagents and reference gas samples

Deionized water, saturated with N_2, was used as carrier fluid in the newly developed analyser. The analytical grade reagents used were pyrogallic acid (Vetec), 1.5 mol/l and potassium hydroxide (Synth), 1–3 mol/l. The primary standards of gas mixtures containing O_2 and N_2 were supplied by White Martins and those containing N_2 and CO_2 by Air Liquide. The mixtures of O_2, CO_2 and N_2 were purchased from both White Martins and Air Liquide. The concentrations of the above gas mixtures are expressed in percentage volume (% v/v), certified by gas chromatography with a degree of precision not greater than or equal to ± 1%.

Operation of the bisegmented flow analyser (BFA)

The basic layout of the analyser for the determination of CO_2 and O_2 levels in the head-space of packages is shown in Figure 1. The analyser is equipped with a proportional injector[11] through which the gas sample is introduced into the system. The sample material is transported by means of a peristaltic pump up to the optical switch OP_1, where the analytical process starts. The working principle of this process is based on the transfer of the target gas type from the sample segment to the liquid phase, while either the variation of the volume of the gaseous segment or the change caused by absorption of the gaseous component being analysed into the liquid segment is used for quantitative determination. The bisegmented profile, shown in detail in Figure 1, is created by filling sampling loop L_5 (200 μl) with the gas sample using the same gastight syringe previously utilized to extract the gas from the head-space of the packages through a septum. Loops L_{1-2} are filled with an inert gas, while loops L_{3-4} are filled with deionized water. The profile of the sample is shown in detail in Figure 1.

When the sample is introduced into the system, the initial time interval (t_i^a) between the passage of the liquid–gas and gas–liquid interfaces of bubble #2 is measured and stored on a microcomputer in a user's file. Next, the logical state of OP_3 is monitored up to the moment when the gas–liquid interface of the first bubble is detected by the optical switch. At this stage, the reagents (KOH and pyrogallic acid) are added through electromechanical three-way valves V_1 and/or V_2 (NResearch-161T031). During the time the valves remain activated, the logical level of OP_2 is monitored up to the moment the liquid–gas interface is detected, that is, when the second bubble reaches OP_2. Upon detection of the second bubble by OP_2, the valves are shut off to assure that the reagents are fed only into the liquid segment. After passing point X, the segment containing the reagent and the bubbles is carried through a glass reactor tube (inside diameter 2 mm). Since glass is a hydrophilic material, a film of aqueous reagent forms on the surface of the wall of the reactor tube. Bubble #2, which contains the gas sample, flows over the reagent film built up by the reagent segment (S_R). The gaseous component to be analysed is absorbed and a contraction of the volume of bubble #2 is observed. The new time interval, t_f^a, is measured by optical switch OP_4. The contraction of the volume of the bubble containing the gas sample serves as the basis for quantifying the O_2 and/or CO_2 levels of samples that contain concentrations above 2% (v/v). Quantification of oxygen levels smaller than 2% (v/v) requires spectrophotometric detection, which is performed immediately after measuring the final time interval of the bubble containing the sample. For this purpose, the spectrophotometric measuring device is activated to analyse the product that results from the reaction between potassium pyrogalate and oxygen— extracted by the collector segment (S_C) — and that immediately follows bubble #2. Upon completion of this stage, the system is ready for a new sample.

The parameter used to represent the quantity of gas component to be analysed present in samples containing levels above 2% (v/v) O_2 or CO_2, will be the following ratio:

Table 1. Determination of O_2 by spectrophotometric bisegmented flow analysis of the head-space of packages containing grated Parmesan cheese, fresh pasta and milk powder

Parmesan cheese $[O_2]$% (v/v)			Fresh pasta $[O_2]$% (v/v)			Milk powder $[O_2]$% (v/v)		
GC[a] (s = ±0.04)	SBFA[b] (s = ±0.03)	Error (v/v) %[c]	GC[a] (s = ±0.08)	SBFA[b] (s = ±0.05)	Error (v/v) %[c]	GC[a] (s = ±0.12)	SBFA[b] (s = ±0.17)	Error (v/v) %[c]
0.06	0.05	−0.01	1.40	0.88	−0.52	1.69	2.48	0.79
0.07	0.01	−0.06	4.39	2.92	−1.47	2.17	2.11	−0.06
0.11	0.09	−0.02	1.91	1.39	−0.52	2.27	2.16	−0.11
0.11	0.07	−0.04	6.67	4.18	−2.49	3.50	3.11	−0.39
0.08	0.03	−0.05	1.79	3.30	1.51	2.74	2.15	−0.59
0.04	0.06	0.02	1.63	1.14	−0.49	2.38	1.96	−0.42
0.08	0.01	−0.07	1.50	1.03	−0.47	2.97	2.90	−0.07

[a] Determination by gas chromatography.
[b] Spectrophotometric bisegmented flow analysis.
[c] Absolute error.
s, estimate of standard deviation for five measurements.

$$\tau_{AN} = \tau_A/\tau_N$$

where: $\tau_A = t_f^a/t_i^a$ is the ratio originated when the gas sample is injected into the system and $\tau_N = t_f^n/t_i^n$ is the ratio originated by the injection of nitrogen.[12] In the case of O_2 levels smaller than 2% (v/v), absorbance will be used as the analytical parameter associated to the O_2 concentration.[13]

The levels of CO_2 are determined with the addition of only KOH, whereas the concentration of O_2 is measured adding both KOH and pyrogallic acid. This can be controlled with an especially programmed microcomputer or simply by filling one of the valves with water instead of pyrogallic acid. If necessary, the CO_2 level determined by the volumetric method may be corrected by subtracting the spectrophotometrically determined O_2 concentration.

Gas chromatography

Samples (300 µl) were drawn from the gas present in the head-space of the packages using a septum and a gas-tight syringe. Next, the sample material was introduced into a gas chromatograph (CG

Table 2. Determination of CO_2 by volumetric bisegmented flow analysis of the head-space of packages containing grated Parmesan cheese, fresh pasta and milk powder

Parmesan cheese $[CO_2]$% (v/v)			Fresh pasta $[CO_2]$% (v/v)			Milk powder $[CO_2]$% (v/v)		
GC[a] (s = ±0.39)	VBFA[b] (s = ±0.66)	Error (v/v) %[c]	GC[a] (s = ±2.00)	VBFA[b] (s = ±0.15)	Error (v/v) %[c]	GC[a] (s = ±0.52)	VBFA[b] (s = ±0.39)	Error (v/v) %[c]
43.68	43.21	−0.47	61.48	60.53	−0.95	6.78	6.77	−0.01
43.52	42.98	−0.54	61.57	59.66	−1.91	6.62	6.58	−0.04
43.87	42.69	−1.18	62.04	62.14	0.10	5.57	4.93	−0.64
43.59	43.20	−0.39	60.84	60.69	−0.15	7.41	6.19	−1.22
42.96	43.02	0.06	60.48	60.10	−0.38	6.90	6.72	−0.18
42.65	42.39	−0.26	60.49	61.40	0.91	5.51	5.99	0.48
43.57	43.67	0.10	61.07	60.22	−0.85	5.70	6.38	0.68

[a] Determination by gas chromatography.
[b] Volumetric bisegmented flow analysis.
[c] Absolute error.
s, estimate of standard deviation for five measurements.

Instrumentos Cientificos, Model 2527) equipped with a thermal conductivity detector set at 150°C, parallel molecular sieve columns 5A and Porapak Q at 83°C and injector at 70°C. The chromatograms were obtained by means of a Shimadzu (Model CR4A) integrator. Analytical curves were used to evaluate the results.

RESULTS AND DISCUSSION

The bisegmented flow analyser (BFA) developed for the determination of O_2 and CO_2 in packages of the investigated foods presents satisfactory accuracy (absolute error smaller than 2.5%, v/v) and precision (relative standard deviation ranging from 0.2% to 8.5%), as indicated by the results presented in Tables 1 and 2. In this analyser, the chemical reactions and the detection take place sequentially and automatically within the glass reactor. The advantage of the system is that dispersion of the reaction product is avoided, since it is collected and kept confined between two bubbles, contributing to adequate sensitivity. The bisegmented flow analyser compares favourably with similar devices to determine CO_2 and O_2 in food packages available on the market, in that the bisegmented flow analyser does not require samples greater than 100–200 µl. Other commercial analysers require sample volumes of 5–30 ml. The use of small sample volumes is especially advantageous when packages with only very limited head-space are to be analysed as to O_2 and CO_2 levels. On the other hand, the commercial analysers respond very quickly to the concentration of the substances being analysed, approximately 5 s, whereas the BFA takes about 2 min to determine both gas types. Despite the increase of the determination time, the BFA is a low-cost alternative for the analysis of gases present in the head-space of packages. In addition to the aspects described above, the BFA is a versatile device, since it can also be used to determine other gaseous constituent elements by substituting appropriate reagents.

CONCLUSION

There is good correlation between the results of the determination of O_2 and CO_2 present in the head-space of food-packages obtained by means of the spectrophotometric and volumetric bisegmented flow system and the results produced by gas chromatography, which is the technique most commonly used for this purpose. For that reason, the device and methodology described in this paper represent a new and valid alternative for the determination of gases in packages.

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