# Optimization of modified atmosphere packaging with respect to physicochemical characteristics of *Requeijão*

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**Abstract**

The effects of modified atmosphere packaging on physicochemical and sensorial characteristics (contents of free fatty acids, lactose, lactic acid and moisture, as well as pH and rigidity) in Portuguese whey cheese (*Requeijão*) were studied following a response surface methodology using storage time, storage temperature and fraction of $CO_2$ in the flushing gas as manipulated variables. Inspection of the sensorial optima in terms of the different parameters indicated that it is convenient to set the storage temperature equal to 4°C because no significant lipolysis takes place, irrespective of overhead atmosphere. Plain $CO_2$ as flushing gas will in general ensure more constant composition until 15 days and will provide protection against extensive lipolysis. In terms of overall visual aspect, all packaged cheeses were preferred to their unpackaged counterparts; however, in terms of acidic smell, only whey cheeses stored at 4°C exhibited significant differences relative to those stored at higher temperatures. 

*Keywords:* Package; Whey cheese; Dairy foods; Technological optimization

## 1. Introduction

The annual worldwide production of whey is of the order of 85 million tons, as a consequence of a cheese production which has been increasing at a steady rate of ca. 3% per year (Zall, 1984). Whey is a dilute liquid that contains lactose, proteins, minerals, and traces of fat and organic acids, which add up to ca. 7% (w/v) of total solids, 75% of which is lactose and 10% of which is whey protein (Mulvihill, 1991). Recovery of proteins from whey is a well established technique in the dairy industry; properties that have been taken advantage of in the manufacture of whey protein concentrates and isolates include differences in molecular sizes, solubility at high temperatures, net charges and polyphosphate-mediated aggregation (Mathews, 1984). Good yields can be obtained by heating sweet whey at pH values between 6.0 and 6.5; however, the precipitate thus formed is rather rigid, a deed that limits its applications (Mathews, 1984; Wingerd, Saperstein & Lutwak, 1970). The preparation of whey cheeses, of the Ricotta type, is a better alternative since denatured whey proteins are consumed as such (Mathur & Shahani, 1981; Modler & Emmons, 1989; Pereira, Póvoa & Cruz, 1982; Pintado, Lopez da Silva & Malcata, 1996; Roseiro & Wilbey, 1991; Vodret, 1970; Weatherup, 1986; Ziino, Salvo, Stagno-d'Alcontres & Chiofalo, 1993). Portuguese whey cheese, called *Requeijão*, is traditionally produced from ovine whey, although it can utilize bovine whey as well (Santiago, 1993). Despite such possibilities, only ca. 2% of the total whey produced in Portugal is at present used to manufacture *Requeijão* (Ferraz, 1998). Owing to its high contents in protein, water and lactose, and to poor handling practices, *Requeijão* is easily contaminated by environmental microorganisms, which bring about physicochemical changes that rapidly constrain acceptance by the final consumer. Such whey cheese is generally transported and sold without a closed package, and exposure in the store stand to outer air without proper sterile conditioning often confines its shelf-life to 2–3 days. Therefore, tailor-made packages specifically designed to improve the quality and safety of whey cheese are in order if expansion of its market is sought. Evidence exists that modified atmosphere packaging can extend the shelf-life of perishable food products between 50 and 400% (Hotchkiss, 1988).

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Fresh cheese, as is the case of *Requeijão*, is one such product with respect to modified atmosphere packaging (Berne, 1994; Fedio, Macleod & Ozimek, 1994; Moir, Eyles & Davery, 1993; Rosenthal, Rosen, Bernstein & Popel, 1991).

The goal of this research effort was thus to assess the effect of several modified atmospheres upon the physicochemical characteristics of *Requeijão*, and to find the composition of the flushing gas that optimizes sensorial quality throughout storage.

## 2. Materials and methods

### 2.1. Preparation and packaging of cheese samples

The whey cheeses were prepared in loco, following the traditional protocol, by heating ovine whey containing ca. 10% (v/v) caprine milk, under constant and soft stirring, to ca. 80°C. Then, the temperature was rapidly raised to 95°C and maintained at that level for 15 min. The floating curd was finally transferred with a scoop into perforated molds and left to drain for ca. 30 min at room temperature. After this period, 32 whey cheeses were closed in individual sterile bags and transported under refrigerated conditions to our premises. Two whey cheeses were analyzed upon receipt (reference whey cheeses), seven whey cheeses were stored unpackaged at 4°C (control whey cheeses used for sensorial analysis) and the remaining 23 whey cheeses were packaged under modified atmospheres according to the factorial design explained below. The samples were packaged into high barrier modified atmosphere Cryovac BB4L bags (Grace, Barcelona, Spain) using a Multivac A300/42 apparatus (Wolfertschwenden, Germany); the flushing gases used include 100 % (v/v) $CO_2$, 100 % (v/v) $N_2$, and a mixture of 50 % (v/v) $CO_2$ and 50 % (v/v) $N_2$. The gases were flushed for a few seconds and the bags sealed immediately thereafter in agreement with the following protocol: a soft vacuum (10 mbar) was initially produced for 2 s, the headspace of the bags was flushed with the appropriate gas at 550 mbar, and automatic sealing took place after 1.5 s; although the evolution of the composition of the overhead atmospheres was monitored, the designation "overhead gas composition" refers to the composition of the mixture used for initial flushing of the cheese bags. Whey cheeses at each storage condition were picked at random, and samples were examined and analyzed at 2, 6, 10 and 15 days, according to the experimental plan detailed in Table 1.

### 2.2. Overhead atmosphere measurements

The gas composition in the headspace of the bags was measured by gas chromatography using a Shimadzu GC-15-A (Kyoto, Japan), with injection of 0.6 ml through a 3 m × 0.32 mm column packed with 80/100 mesh Carbosieve S II (Supelco, Belfont PA, USA) and detection by thermal conductivity. The injector and detector temperatures were set at 120 and 210°C, respectively. The temperature of the column oven was 40°C for 6 min, 15°C $min^{-1}$ up to 170°C, and 170°C for 5 min. The carrier gas was helium at 30 ml/min. The bridge-current for the detector was 140 mA.

### 2.3. Chemical analyses

The dry weight of the whey cheeses was determined according to the IDF method (IDF, 1958). The pH on the surface and in the bulk of the whey cheese was measured with a potentiometer Crison Microph 2001 (Barcelona, Spain). Lactose and lactic acid were quantified by HPLC in the same run, using calibration curves previously prepared with chromatographic standards and an apparatus from Beckman Instruments (Fullerton CA, USA) with an Aminex HPX-87X cation exchange column from BioRad (Richmond CA, USA); a flow rate of 0.5 ml $min^{-1}$ of 0.005 N $H_2SO_4$ (Merck, Frankfurt, Germany) was employed as eluant, and detection was by refractive index at 30°C for lactose and UV absorbance for lactic acid. Prior to analysis, all samples were pretreated as follows: 10 g of sample was homogenized with 30 ml of a 0.5 M solution of perchloric acid (Merk) for 3 min in a Stomacher Lab Blender 400 (Seward Medical, London, UK), allowed to stand for 2 h at a refrigerated temperature in a closed vessel and filtered through a 0.22 μm membrane Syrfil filter (Nucleopore, Cambridge MA, USA). Most samples were replicated so as to estimate the experimental variability, which was expressed in the form of an average standard error for each data set.

Quantitative extraction of free fatty acids (FFA) was achieved with diethylether, according to Nunez, Garcia-Aser, Rodriguez-Martin, Medina and Gaya (1986), using 6-g samples of whey cheese. Quantification of FFA was performed following the method by Garcia, Reyes, Malcata, Hill and Amundson (1990), later modified by Balcão and Malcata (1998), using *p*-bromophenacylbromide (Sigma, St. Louis MO, USA), 18-crown-6-ether (Sigma) and potassium carbonate (Merk) as derivatization agents, and formic acid (Romil, Leicester, UK) as quenching agent. Nonanoic acid (6.4 mM) and heptadecanoic acid (11.13 mM) were used as internal standards. To 1.5 ml of FFA extracts, selected volumes of solution of internal standards (ranging from 0.1 to 0.6 ml, depending on the expected concentration of the sample) were added prior to derivatization. Separation was effected with a C-18 Reverse Phase column (from Beckman, San Roman CA, USA) at 33°C using 5 % (v/v) acetonitrile (from Romil) in methanol and 5 % (v/v)

acetonitrile in water as solvents for the mobile phase gradient, and 20 μl as injection volume; detection was by absorbance at 254 nm.

### 2.4. Rheological measurements

An Instron Puncture Tester model 4501 (High Wycombe, UK) was used to produce the force–displacement curves of the whey cheeses. The puncture was effected with a plunger with 5 mm of diameter, at a constant penetration rate of 20 mm/min and for a fixed height of 25 mm. Six determinations per cheese were made at room temperature (23°C). The penetration analyses were chosen because they yielded more reproducible results than compression tests, and because they have also been shown to correlate well with sensory analyses in what concerns firmness and rigidity (Brennan, Jowitt & Williams, 1975). The slope of the rupture curve was selected as the best analytical parameter since it is generally claimed to be a measure of the rigidity of the sample (Zoon, 1991).

### 2.5. Sensorial assessments

Unpackaged whey cheeses maintained at 4°C (i.e. at preservation conditions that mimic general practice) have often a limited shelf-life (ca. 3 days), probably owing to airborne microbial contaminants and water loss (which makes overall aspect decay, and hence constrains demand of the product by potential consumers). The chemical composition of a control whey cheese, maintained unpackaged under similar storage conditions (denoted as reference), is depicted in Table 1a and b; inspection of this table indicates that by 6 days of storage, there is a significant decrease in pH and moisture, as well as an increase in free fatty acid content. The microbiological monitoring of said whey cheese (Pintado & Malcata, 2000) showed even more extensive

Table 1a
Second order experimental design using incubation time, temperature and flushing gas composition as manipulated variables, with experimental data for inner and outer pH, moisture content, rupture slope, and lactose and lactic acid contents[a]

| | | Experimental design | | | Physicochemical parameters | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | U | Time (days) | Temperature (°C) | Overhead gas | Outer pH | Inner pH | Slope (N/mm) | Moisture (%) | Lactose (mg/g) | Lactic acid (mg/g) |
| | Reference | 0 | 4 | Without package | 6.18 | 6.66 | 0.09 | 72.08 | 34.15 | 4.95 |
| | Reference | 2 | 4 | Without package | 6.26 | 6.70 | 0.13 | 72.65 | 37.95 | 4.54 |
| | Reference | 6 | 4 | Without package | 5.50 | 6.50 | 0.18 | 66.62 | 45.09 | 4.94 |
| | Reference | 10 | 4 | Without package | 5.40 | 6.10 | 0.19 | 65.77 | 57.74 | 2.65 |
| | Reference | 15 | 4 | Without package | 4.78 | 4.79 | 0.78 | 53.37 | 73.54 | 2.39 |
| | 1 | 2 | 4 | 100% $CO_2$ | 6.02 | 6.22 | 0.12 | 71.25 | 32.94 | 4.94 |
| | 2 | 2 | 4 | 100% $N_2$ | 5.69 | 6.43 | 0.18 | 72.30 | 33.47 | 4.41 |
| | 3 | 2 | 18 | 100% $CO_2$ | 5.92 | 6.21 | 0.10 | 73.45 | 37.17 | 4.19 |
| | 4 | 2 | 18 | 100% $N_2$ | 5.50 | 5.90 | 0.16 | 73.49 | 33.40 | 4.31 |
| | 5 | 6 | 4 | 100% $CO_2$ | 6.10 | 6.15 | 0.08 | 73.66 | 33.88 | 4.82 |
| C | 6 | 6 | 4 | 100% $N_2$ | 5.80 | 6.59 | 0.10 | 73.02 | 34.14 | 4.49 |
| | 7 | 6 | 18 | 100% $CO_2$ | 5.40 | 5.33 | 0.17 | 71.46 | 29.27 | 7.85 |
| | 8 | 6 | 18 | 100% $N_2$ | 5.05 | 5.67 | 0.10 | 72.52 | 29.26 | 4.97 |
| | 9 | 15 | 4 | 100% $CO_2$ | 5.77 | 5.99 | 0.10 | 73.88 | 33.22 | 3.84 |
| | 11 | 15 | 4 | 100% $N_2$ | 5.22 | 6.34 | 0.11 | 73.40 | 34.53 | 1.92 |
| | 10 | 15 | 18 | 100% $CO_2$ | 4.15 | 4.40 | 0.15 | 72.84 | 25.03 | 12.63 |
| | 12 | 15 | 18 | 100% $N_2$ | 4.18 | 4.52 | 0.14 | 73.33 | 19.46 | 14.61 |
| c | 13 | 10 | 12 | 50% $N_2$ + 50% $CO_2$ | 4.47 | 5.30 | 0.13 | 74.11 | 22.84 | 7.17 |
| | 14 | 10 | 12 | 50% $N_2$ + 50% $CO_2$ | 4.50 | 5.03 | 0.18 | 74.05 | 21.57 | 8.03 |
| | 15 | 2 | 12 | 50% $N_2$ + 50% $CO_2$ | 6.08 | 6.34 | 0.13 | 70.12 | 33.40 | 5.14 |
| | 16 | 6 | 12 | 50% $N_2$ + 50% $CO_2$ | 5.05 | 6.03 | 0.09 | 72.00 | 27.08 | 5.63 |
| | 17 | 15 | 12 | 50% $N_2$ + 50% $CO_2$ | 4.66 | 5.10 | 0.12 | 72.55 | 25.34 | 8.13 |
| | 18 | 10 | 4 | 50% $N_2$ + 50% $CO_2$ | 5.60 | 6.28 | 0.08 | 71.84 | 32.78 | 3.26 |
| ax | 19 | 10 | 4 | 50% $N_2$ + 50% $CO_2$ | 5.62 | 6.25 | 0.10 | 73.17 | 31.67 | 3.51 |
| | 20 | 10 | 18 | 50% $N_2$ + 50% $CO_2$ | 4.65 | 4.62 | 0.12 | 73.11 | 25.78 | 12.93 |
| | 21 | 10 | 12 | 100% $CO_2$ | 4.30 | 5.50 | 0.11 | 72.70 | 29.08 | 7.77 |
| | 22 | 10 | 12 | 100% $N_2$ | 4.62 | 4.83 | 0.09 | 73.70 | 23.43 | 11.46 |
| | 23 | 10 | 12 | 100% $N_2$ | 4.52 | 4.82 | 0.11 | 71.79 | 26.88 | 10.66 |
| Average standard error | | | | | 0.0011 | 0.0074 | 0.0004 | 0.5421 | 1.4735 | 0.1452 |

[a] ax, axial points; c, center points; C, corner points; U, rUn.

Table 1b
Second order experimental design using incubation time, temperature and flushing gas composition as manipulated variables, with experimental data for each free fatty acid and total free fatty acids (TFFA) contents[a]

| | | Experimental design | | | Individual fatty acids | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | U | Time (days) | Temperature (°C) | Overhead gas | $C_{4:0}$ (mg/g) | $C_{6:0}$ (mg/g) | $C_{8:0}$ (mg/g) | $C_{10:0}$ (mg/g) | $C_{12:0}$ (mg/g) | $C_{14:0}$ (mg/g) | $C_{16:0}$ (mg/g) | $C_{18:0}$ (mg/g) | $C_{18:1}$ (mg/g) | $C_{18:2}$ (mg/g) | $C_{18:3}$ (mg/g) | TFFA (mg/g) |
| | Reference | 0 | 4 | Without package | 0.024 | 0.026 | 0.033 | 0.042 | 0.043 | 0.056 | 0.065 | 0.070 | 0.070 | 0.066 | 0.000 | 0.498 |
| | Reference | 0 | 4 | Without package | 0.021 | 0.026 | 0.033 | 0.042 | 0.047 | 0.058 | 0.064 | 0.066 | 0.072 | 0.066 | 0.000 | 0.494 |
| | Reference | 0 | 4 | Without package | 0.021 | 0.030 | 0.033 | 0.042 | 0.046 | 0.052 | 0.061 | 0.066 | 0.074 | 0.063 | 0.061 | 0.549 |
| | Reference | 0 | 4 | Without package | 0.163 | 0.214 | 0.274 | 0.415 | 0.382 | 0.396 | 0.471 | 0.455 | 0.726 | 0.469 | 0.412 | 4.377 |
| | Reference | 0 | 4 | Without package | 0.268 | 0.325 | 0.400 | 0.648 | 0.556 | 0.566 | 0.765 | 0.566 | 1.163 | 0.598 | 0.476 | 6.331 |
| | 1 | 2 | 4 | 100% $CO_2$ | 0.033 | 0.026 | 0.033 | 0.043 | 0.047 | 0.057 | 0.065 | 0.069 | 0.069 | 0.065 | 0.000 | 0.506 |
| | 2 | 2 | 4 | 100% $N_2$ | 0.020 | 0.025 | 0.032 | 0.042 | 0.047 | 0.058 | 0.065 | 0.071 | 0.074 | 0.068 | 0.062 | 0.566 |
| | 3 | 2 | 18 | 100% $CO_2$ | 0.022 | 0.025 | 0.033 | 0.043 | 0.046 | 0.056 | 0.063 | 0.068 | 0.071 | 0.064 | 0.000 | 0.491 |
| | 4 | 2 | 18 | 100% $N_2$ | 0.030 | 0.028 | 0.039 | 0.055 | 0.051 | 0.063 | 0.071 | 0.074 | 0.088 | 0.070 | 0.066 | 0.635 |
| | 5 | 6 | 4 | 100% $CO_2$ | 0.027 | 0.026 | 0.033 | 0.042 | 0.047 | 0.058 | 0.064 | 0.064 | 0.070 | 0.065 | 0.000 | 0.494 |
| C | 6 | 6 | 4 | 100% $N_2$ | 0.026 | 0.026 | 0.033 | 0.043 | 0.047 | 0.053 | 0.063 | 0.064 | 0.071 | 0.063 | 0.061 | 0.549 |
| | 7 | 6 | 18 | 100% $CO_2$ | 0.028 | 0.026 | 0.032 | 0.041 | 0.046 | 0.057 | 0.062 | 0.067 | 0.069 | 0.065 | 0.000 | 0.494 |
| | 8 | 6 | 18 | 100% $N_2$ | 0.161 | 0.161 | 0.178 | 0.217 | 0.166 | 0.160 | 0.191 | 0.169 | 0.263 | 0.151 | 0.136 | 1.952 |
| | 9 | 15 | 4 | 100% $CO_2$ | 0.020 | 0.026 | 0.034 | 0.047 | 0.048 | 0.061 | 0.067 | 0.072 | 0.074 | 0.067 | 0.000 | 0.516 |
| | 11 | 15 | 4 | 100% $N_2$ | 0.024 | 0.027 | 0.035 | 0.049 | 0.049 | 0.062 | 0.069 | 0.074 | 0.083 | 0.070 | 0.000 | 0.543 |
| | 10 | 15 | 18 | 100% $CO_2$ | 0.030 | 0.026 | 0.033 | 0.043 | 0.048 | 0.055 | 0.065 | 0.071 | 0.072 | 0.064 | 0.000 | 0.505 |
| | 12 | 15 | 18 | 100% $N_2$ | 0.104 | 0.131 | 0.172 | 0.250 | 0.227 | 0.270 | 0.333 | 0.314 | 0.461 | 0.314 | 0.265 | 2.840 |
| c | 13 | 10 | 12 | 50% $N_2$ + 50% $CO_2$ | 0.138 | 0.174 | 0.236 | 0.349 | 0.326 | 0.359 | 0.433 | 0.395 | 0.677 | 0.407 | 0.341 | 3.835 |
| | 14 | 10 | 12 | 50% $N_2$ + 50% $CO_2$ | 0.106 | 0.133 | 0.172 | 0.221 | 0.240 | 0.286 | 0.329 | 0.354 | 0.368 | 0.336 | 0.317 | 2.862 |
| | 15 | 2 | 12 | 50% $N_2$ + 50% $CO_2$ | 0.020 | 0.026 | 0.033 | 0.043 | 0.048 | 0.060 | 0.067 | 0.071 | 0.072 | 0.069 | 0.000 | 0.509 |
| | 16 | 6 | 12 | 50% $N_2$ + 50% $CO_2$ | 0.033 | 0.026 | 0.034 | 0.045 | 0.048 | 0.057 | 0.066 | 0.073 | 0.080 | 0.065 | 0.000 | 0.529 |
| | 17 | 15 | 12 | 50% $N_2$ + 50% $CO_2$ | 0.133 | 0.177 | 0.214 | 0.275 | 0.288 | 0.335 | 0.388 | 0.417 | 0.451 | 0.398 | 0.377 | 3.453 |
| | 18 | 10 | 4 | 50% $N_2$ + 50% $CO_2$ | 0.027 | 0.026 | 0.033 | 0.041 | 0.047 | 0.058 | 0.065 | 0.071 | 0.070 | 0.066 | 0.000 | 0.503 |
| ax | 19 | 10 | 4 | 50% $N_2$ + 50% $CO_2$ | 0.025 | 0.026 | 0.033 | 0.043 | 0.047 | 0.058 | 0.065 | 0.070 | 0.070 | 0.068 | 0.000 | 0.503 |
| | 20 | 10 | 18 | 50% $N_2$ + 50% $CO_2$ | 0.136 | 0.165 | 0.214 | 0.279 | 0.297 | 0.351 | 0.417 | 0.429 | 0.472 | 0.397 | 0.373 | 3.530 |
| | 21 | 10 | 12 | 100% $CO_2$ | 0.029 | 0.026 | 0.033 | 0.041 | 0.047 | 0.055 | 0.064 | 0.069 | 0.070 | 0.064 | 0.000 | 0.050 |
| | 22 | 10 | 12 | 100% $N_2$ | 0.254 | 0.154 | 0.199 | 0.255 | 0.287 | 0.348 | 0.391 | 0.426 | 0.438 | 0.411 | 0.267 | 3.429 |
| | 23 | 10 | 12 | 100% $N_2$ | 0.131 | 0.171 | 0.217 | 0.285 | 0.296 | 0.337 | 0.392 | 0.418 | 0.462 | 0.399 | 0.377 | 3.483 |
| Average standard error | | | | | 0.0016 | 0.0002 | 0.0004 | 0.0174 | 0.0007 | 0.0005 | 0.0011 | 0.0002 | 0.0096 | 0.0005 | 0.0012 | 0.0948 |

[a] ax, axial points; c, center points; C, corner points; U, run.

alterations, with substantial increases of viable counts of several microbial groups. On each day of sampling, whey cheeses were evaluated by 19 panelists that are familiar with *Requeijão*. Whey cheeses were compared with a control whey cheese (viz. *Requeijão* prepared under similar conditions but stored unpackaged at 4°C under a stagnant atmosphere with regular composition; this control imitated as much as possible *Requeijão* when lying on commercial stands and stored under regular conditions) in terms of acid smell, brightness of surface and overall aspect, based on a multiple comparison test (Meilgaard, Civille & Carr, 1988). The scales used for both parameters ranged from 1 to 9 (1 — "extremely less than control"; 5 — "equal to control"; 9 — "extremely more than control"). A replicated sample was included in each sensory test to assess inter-panelist variability.

### 2.6. Mathematical modeling

The first empiric model that was tentatively fitted, by linear regression, to the first 14 experimental data listed in Table 1 had the form

$$\hat{y} = \bar{y} + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_3 x_3 \quad (1)$$

where $\hat{y}$ is the fitted response, $\bar{y}$ is the average of all data and the $\alpha$s are adjustable parameters. The $x_i$ s are the manipulated technological variables under scrutiny, expressed in coded (normalized) form, and are defined as: $x_1 = (t-10)/8$, where $t$ is the storage time expressed in days; $x_2 = (T-12)/8$, where $T$ is the storage temperature, expressed in °C; and $x_3 = (g-50)/50$, where $g$ is the fraction of $N_2$ used as flushing gas in each package (in a binary mixture also containing $CO_2$). The estimates of the sums of all quadratic effects associated with the aforementioned linear model form (results not shown), obtained as outlined elsewhere (Box, Hunter & Hunter, 1978), indicated that second order effects are likely important; hence, the experimental design was expanded and an extra nine experimental points (laid out as axial points) were generated; these extra data are depicted in Table 1 as the last nine data. The model to be fitted by linear regression analysis to the data was then

$$\hat{y} = \bar{y} + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \ldots$$
$$\ldots \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \ldots$$
$$\ldots \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad (2)$$

where the $\beta$s are adjustable parameters.

Differentiation of Eq. (2) independently with respect to $x_1$, to $x_2$ and to $x_3$, and setting of the result obtained equal to zero, provides (as a whole) the necessary condition for a local optimum to exist. The resulting three linear equations can, in turn, be algebraically solved with respect to $x_1$, $x_2$ and $x_3$, respectively; the results in terms of optimum loci when variables $x_2$ and $x_3$, or $x_1$ and $x_3$, or $x_1$ and $x_2$, respectively, were deliberately prefixed are tabulated in Table 3. The sign of the effect estimated ($\beta_{11}$, $\beta_{22}$ or $\beta_{33}$) gives immediate indication whether the locus is a maximum (−) or a minimum (+), as a consequence of the definition of the second order derivative.

The experimental results pertaining to sensorial assessment were subject to analyses of variance (ANOVA). Tukey's test (Box et al., 1978) was applied to find those data that bear a significant difference between themselves.

## 3. Results and discussion

### 3.1. Evolution of overhead atmosphere

The headspace atmosphere did not undergo significant changes in composition throughout storage time, except for storage at 18°C under plain $N_2$. In this case, there was a continuous increase in the fraction of $CO_2$ (from 0 to ca. 15 % (v/v) by 15 days), likely as a result of the high microbial growth, as discussed below. Hence, the conclusions based on assumption of an overhead atmosphere that guarantees environmental conditions identical to those prevailing initially is a good approximation, especially for storage temperatures more likely to be encountered in practice.

### 3.2. Physicochemical considerations

The second order experimental design, as well as the experimental results obtained for the reference whey cheeses, the 12 corner points, the two center points and the nine axial points, are all depicted in Table 1 in terms of the physicochemical parameters measured. The experimental results pertaining to initial stages of storage are similar to those obtained for Ricotta cheeses in terms of pH (Cavaliere, 1988), as well as in terms of contents of moisture, lactose and FFA (Ziino et al., 1993); however, the relative proportion of each FFA shows slight differences, which were naturally expected in view of the different breeds considered. Inspecting of the pH data, it is notorious that the inner pH did not undergo significant variation when storage was at 4°C (i.e. it never decreased below 6 for any of the packaging atmospheres tested); the outer pH, despite its higher decrease when nitrogen is present, was never above 5.2. On the other hand, storage above refrigeration temperatures, i.e. 12 or 18°C, led to important, decreases in the values of both the inner and outer pH by 2 days, for all flushing gas compositions experimented. These results confirm that a temperature control is important, especially at the last stages of storage if effective packaging of whey cheese were sought.

The results for the rupture slope and for the moisture content did not undergo major variations, which confirms the absence of changes in rigidity of whey cheeses as losses of water are not allowed; this is an important advantage when comparing packaged with unpackaged whey cheese, because the latter usually loses its freshness fast owing to unconstrained dehydration. Moir et al. (1993) have claimed that packaging under plain $CO_2$ affects texture of cheese and leads to development of a slightly fizzy characteristic in one's mouth, even a spongy sensation (Berne, 1994); although the experimental whey cheeses were not tasted by the panel for safety reasons, their cut and touch did not unfold any of these characteristics, probably owing to the high moisture content of these whey cheeses. The package collapse referred to in the literature for high concentrations of $CO_2$ (Hotchkiss, 1988) was also not observed in the cheeses studied, probably owing to their lower fat content and shorter storage periods.

In terms of lactose, no variation at 4°C was observed for any of the overhead atmospheres tested; however, lactic acid exhibited a slight decrease in concentration over 6 days, which can be attributed to the catabolism of lactic acid, as observed in other packaged cheeses (Fedio, Ozimek & Wolfe, 1994). Our results showed that, at higher storage temperatures, lactose was actually metabolized and formation of lactic acid was consequently observed; the rate of such metabolism was slightly affected by the concentration of $CO_2$ in the overhead atmosphere.

Our whey cheeses were rich in saturated FFA, especially $C_{16:0}$ and $C_{18:0}$ and, to a lesser extent, $C_{14:0}$; the predominant unsaturated FFA were $C_{18:1}$ and $C_{18:2}$. No significant lipolysis took place in whey cheese stored at 4°C, irrespective of the overhead atmosphere; in the case of plain $CO_2$, no lipolysis was observed for any storage period. All whey cheeses stored under plain $N_2$ and under the equimolar mixture of $N_2$ and $CO_2$, at temperatures other than 4°C, showed lipolysis from 6 days of incubation on, with values similar by 10 days. The prevention of significant lipolysis and dehydration probably accounts for the most important improvement in our study brought about by modified atmosphere packaging. These results suggest that temperature and

$CO_2$ are independently strong inhibitors of microbial-mediated lipolysis in *Requeijão*.

### 3.3. Mathematical modeling

The estimates of all parameters in the model denoted as Eq. (2) are tabulated in Table 2, together with their 95% confidence intervals. Inspection of these values allows one to state that the total FFA content of whey cheese was affected positively by storage time, storage temperature and fraccion of $CO_2$ in overhead atmosphere (mainly via its linear effect) and negatively by the same parameters (mainly via their quadratic effects). All interactions are significant at the 5% level. Hence, our results indicate that increasing storage temperature, increasing storage time or increasing percentage of $N_2$ increase the FFA content of whey cheese; this is consistent with the claim that the effectiveness of MAP decreases as storage temperature increases (Hotchkiss, 1988), mainly as a consequence of microbial growth (which may be implicated in lipolysis). The release of each FFA was affected in a similar fashion, except $C_{4:0}$ (which was only affected negatively by storage time and storage temperature via their quadratic effects), $C_{10:0}$ (which was not affected by flushing gas composition, via its quadratic effect, neither was it by all interactions between factors), $C_{18:1}$ (which was not affected by storage temperature via its linear effect and by storage time and flushing gas composition via their quadratic effects, neither was it by all interactions) and $C_{18:3}$ (which was not affected by interactions storage time/flushing gas composition and storage temperature/overhead gas composition).

Regarding all FFA there is a true local maximum for their concentration (see Table 3), except in what pertains to $C_{4:0}$; most loci are in fact saddle points, thus indicating that the maxima lie on experimental constrains. Nevertheless, in attempts to extend the shelf-life of whey cheeses, lipolysis should be avoided as far as possible in order to reduce decay in flavor and taste; therefore, the objective function would be to minimize release of each FFA. In view of previous discussion, the procedure to find the best conditions would be to set each variable equal to its global minimum or maximum limit (as given by physical or experimental constraints) in order to produce a minimum value of $\hat{y}$ as close as possible to the concentration in the reference *Requeijão*. Whenever necessary, the following assumptions were taken: the storage temperature ($x_1$) was pre-fixed at 4°C (because this is the common temperature for preservation of perishable foods) and the flushing gas composition was left unconstrained in the interval $-1 \leqslant x_3 \leqslant 1$; these assumptions produce the optimum $\hat{y}$ displayed in

Table 2
Values of linear effects ($\beta_1$, $\beta_2$ and $\beta_3$), quadratic effects ($\beta_{11}$, $\beta_{22}$ and $\beta_{33}$) and second-order interactions ($\beta_{12}$, $\beta_{13}$ and $\beta_{23}$) with associated 95% confidence intervals (runs were made in random order), obtained for individual free fatty acid contents, total free fatty acids (TFFA) content, inner pH, outer pH, moisture content, rupture slope, and lactose and lactic acid contents

| Parameter | Grand average | ASE | E&I[a] | | | | | | ASE | | | | ASE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $\beta_1$ | $\beta_2$ | $\beta_3$ | $\beta_{11}$ | $\beta_{22}$ | $\beta_{33}$ | | $\beta_{12}$ | $\beta_{13}$ | $\beta_{23}$ | |
| $C_{4:0}$ | 0.110 | ±0.026 | 0.007 | 0.020 | 0.035 | −0.058 | −0.050 | 0.011 | ±0.009 | 0.018 | 0.015 | 0.023 | ±0.040 |
| $C_{6:0}$ | 0.122 | ±0.009 | 0.023 | 0.029 | 0.035 | −0.039 | −0.041 | −0.138 | ±0.012 | 0.022 | 0.017 | 0.024 | ±0.014 |
| $C_{8:0}$ | 0.154 | ±0.013 | 0.039 | 0.033 | 0.045 | −0.024 | −0.059 | −0.022 | ±0.018 | 0.029 | 0.023 | 0.029 | ±0.021 |
| $C_{10:0}$ | 0.210 | ±0,027 | 0.045 | 0.047 | 0.059 | −0.059 | −0.073 | −0.029 | ±0.035 | 0.043 | 0.034 | 0.039 | ±0.041 |
| $C_{12:0}$ | 0.219 | ±0.018 | 0.045 | 0.038 | 0.055 | −0.059 | −0.087 | −0.029 | ±0.023 | 0.044 | 0.034 | 0.031 | ±0.027 |
| $C_{14:0}$ | 0.253 | ±0.015 | 0.057 | 0.041 | 0.062 | −0.060 | −0.102 | −0.034 | ±0.020 | 0.052 | 0.043 | 0.034 | ±0.023 |
| $C_{16:0}$ | 0.296 | ±0.021 | 0.069 | 0.054 | 0.075 | −0.071 | −0.114 | −0.044 | ±0.028 | 0.066 | 0.052 | 0.042 | ±0.033 |
| $C_{18:0}$ | 0.316 | ±0.009 | 0.074 | 0.031 | 0.071 | −0.058 | −0.176 | −0.028 | ±0.011 | 0.063 | 0.050 | 0.038 | ±0.041 |
| $C_{18:1}$ | 0.408 | ±0.063 | 0.093 | 0.069 | 0.096 | −0.084 | −0.168 | −0.085 | ±0.083 | 0.088 | 0.067 | 0.059 | ±0.027 |
| $C_{18:2}$ | 0.295 | ±0.015 | 0.071 | 0.042 | 0.071 | −0.064 | −0.130 | −0.038 | ±0.019 | 0.064 | 0.052 | 0.036 | ±0.023 |
| $C_{18:3}$ | 0.237 | ±0.023 | 0.065 | 0.056 | 0.081 | −0.056 | −0.106 | −0.051 | ±0.030 | 0.073 | 0.031 | 0.036 | ±0.033 |
| TFFA | 2.570 | ±0.199 | 0.573 | 0.482 | 1.688 | −0.673 | −1.027 | −0.348 | ±0.261 | 0.563 | 0.420 | 0.390 | ±0.305 |
| Inner pH | 5.2906 | ±0.056 | −0.578 | −0.660 | 0.003 | 0.336 | 0.288 | −0.143 | ±0.073 | −0.524 | 0.008 | −0.095 | ±0.085 |
| Outer pH | 4.687 | ±0.022 | −0.602 | −0.388 | −0.094 | 0.387 | 0.573 | −0.153 | ±0.029 | −0.419 | 0.077 | 0.040 | ±0.033 |
| Moisture | 72.594 | ±0.078 | 0.503 | 0.084 | 0.003 | −0.454 | 0.579 | 0.175 | ±0.625 | −0.478 | −0.205 | 0.144 | ±0.729 |
| Slope | 0.114 | ±0.012 | 0.007 | 0.018 | 0.001 | 0.034 | 0.002 | −0.006 | ±0.016 | 0.164 | −0.153 | −0.113 | ±0.019 |
| Lactose | 24.983 | ±0.780 | −3.532 | −3.190 | −1.014 | 2.751 | 3.492 | 1.038 | ±1.030 | −4.624 | −0.392 | −1.115 | ±1.201 |
| Lactic acid | 7.229 | ±0.246 | 2.402 | 3.496 | 0.242 | −1.473 | −1.232 | 0.814 | ±0.323 | 4.185 | 0.426 | 0.253 | ±0.377 |

[a] E&I, effects (linear and quadratic) and interactions (second order); ASE, average standard error; $\beta_1$ effect of normalized value of incubation time, defined as $(t-10)/8$, where $t$ is expressed in days; $\beta_2$, effect of normalized temperature, defined as $(T-12)/8$, where $T$ is expressed in °C; $\beta_3$, effect of normalized flushing gas composition, defined as $(g-50)/50$, where $g$ is the volumetric fraction of $N_2$ in the $CO_2 + N_2$ mixture expressed in %(v/v).

Table 3
Loci and type of optima regarding each of the operating variables storage time ($x_1$), storage temperature ($x_2$) and flushing gas composition ($x_3$) associated with the quadratic models fitted to the data regarding individual free fatty acid contents, total free fatty acid (TFFA) content, inner pH, outer pH, dry weight, rupture slope, and lactose and lactic acid contents[a]

| | LO | | | TO | | | MM | | |
|---|---|---|---|---|---|---|---|---|---|
| Media | $x_1$ | $x_2$ | $x_3$ | $x_1$ | $x_2$ | $x_3$ | Time (days) | Temperature (°C) | Fraction of $N_2$ (%) |
| $C_{4:0}$ | $0.063+0.157\ x_2+0.132\ x_3$ | $0.199+0.183\ x_1+0.232\ x_3$ | $-1.557-0.686\ x_1-1.031\ x_2$ | Max | Max | Min | 8.9 | 10.93 | < 0 |
| $C_{6:0}$ | $0.289+0.228\ x_2+0.211\ x_3$ | $0.356+0.277\ x_1+0.300\ x_3$ | $0.313+0.060\ x_1+0.088\ x_2$ | Max | Max | Max | 14.11 | 16.94 | 69.90 |
| $C_{8:0}$ | $0.794+0.615\ x_2+0.473\ x_3$ | $0.279+0.252\ x_1+0.247\ x_3$ | $0.985+0.509\ x_1+0.648\ x_2$ | Max | Max | Max | > 15 | > 18 | > 100 |
| $C_{10:0}$ | $0.379+0.362\ x_2+0.286\ x_3$ | $0.322+0.294\ x_1+0.269\ x_3$ | $1.035+0.589\ x_1+0.685\ x_2$ | Max | Max | Max | > 15 | > 18 | > 100 |
| $C_{12:0}$ | $0.383+0.377\ x_2+0.292\ x_3$ | $0.217+0.255\ x_1+0.179\ x_3$ | $0.948+0.597\ x_1+0.543\ x_2$ | Max | Max | Max | > 15 | > 18 | > 100 |
| $C_{14:0}$ | $0.467+0.429\ x_2+0.352\ x_3$ | $0.200+0.255\ x_1+0.167\ x_3$ | $0.907+0.688\ x_1+0.497\ x_2$ | Max | Max | Max | > 15 | > 18 | > 100 |
| $C_{16:0}$ | $0.488-0.468\ x_2+0.363\ x_3$ | $0.238+0.293\ x_1+0.181\ x_3$ | $0.850+0.586\ x_1+0.468\ x_2$ | Max | Max | Max | > 15 | 17.97 | > 100 |
| $C_{18:0}$ | $0.643+0.541\ x_2+0.435\ x_3$ | $0.088+0.177\ x_1+0.107\ x_3$ | $1.282+0.705\ x_1+0.679\ x_2$ | Max | Max | Max | > 15 | > 18 | > 100 |
| $C_{18:1}$ | $0.555+0.522\ x_2+0.399\ x_3$ | $0.205+0.262\ x_1+0.175\ x_3$ | $0.568+0.396\ x_1+0.346\ x_2$ | Max | Max | Max | > 15 | > 18 | > 100 |
| $C_{18:2}$ | $0.557+0.499\ x_2+0.408\ x_3$ | $0.160+0.244\ x_1+0.138\ x_3$ | $0.939+0.686\ x_1+0.472\ x_2$ | Max | Max | Max | > 15 | > 18 | > 100 |
| $C_{18:3}$ | $0.579+0.652\ x_2+0.280\ x_3$ | $0.264+0.343\ x_1+0.167\ x_3$ | $0.793+0.308\ x_1+0.349\ x_2$ | Max | Max | Max | > 15 | > 18 | > 100 |
| TFFA | $0.426+0.418\ x_2+0.312\ x_3$ | $0.235+0.274\ x_1+0.190\ x_3$ | $0.989+0.603\ x_1+0.561\ x_2$ | Max | Max | Max | > 15 | > 18 | > 100 |
| Inner pH | $0.849+0.779\ x_2-0.012\ x_3$ | $1.146+0.949\ x_1+0.165\ x_3$ | $0.009+0.029\ x_1-0.331\ x_2$ | Min | Min | Max | > 15 | > 18 | < 0 |
| Outer pH | $0.778+0.546\ x_2-0.099\ x_3$ | $0.339+0.367\ x_1-0.035\ x_3$ | $-0.306+2.518\ x_1+0.130\ x_2$ | Min | Min | Max | > 15 | 16.96 | > 100 |
| Dry weight | $0.556-0.527\ x_2-0.223\ x_3$ | $-0.072+0.413\ x_1-0.124\ x_3$ | $-0.007+0.584\ x_1-0.411\ x_2$ | Max | Min | Min | 13.67 | 12.72 | 61.20 |
| Slope | $-0.099-2.377\ x_2+2.217x_3$ | $-5.000-45.560\ x_1+31.992\ x_3$ | $0.124-12.540\ x_1-9.262\ x_2$ | Min | Min | Max | 9.64 | 12.52 | 54.68 |
| Lactose | $0.643+0.840\ x_2+0.071\ x_3$ | $0.458+0.663\ x_1+0.161\ x_3$ | $0.489+0.189\ x_1+0.538\ x_2$ | Min | Min | Min | > 15 | > 18 | > 100 |
| Lactic acid | $0.816+1.421\ x_2+0.145\ x_3$ | $0.142+1.697\ x_1+0.103\ x_3$ | $0.149-0.262\ x_1-0.155\ x_2$ | Max | Max | Min | 3.31 | 2.24 | 77.85 |

[a] LO, loci of optima; MM, minimum of maxima, minimum of minima, maximum of minima, or maximum of maximum; TO, type of optimum. $x_1$, normalized value of incubation time, defined as $(t-10)/8$, where $t$ is expressed in days; $x_2$, normalized temperature, defined as $(T-12)/8$, where $T$ is expressed in °C; $x_3$, normalized flushing gas composition, defined as $(g-50)/50$, where $g$ is the volumetric fraction of $N_2$ in the $CO_2+N_2$ mixture expressed in %(v/v).

Table 4
Loci, type of optimum and calculated value obtained for the contents of each free fatty acid, total free fatty acids (TFFA) and lactose, and value of inner pH[a,b]

| Parameter | Storage time (days) | $CO_2$ in flushing gas [% (v/v)] | TO | Reference value |
|---|---|---|---|---|
| $C_{4:0}$ (mg/g) | 0 | 91 | | |
| | 6 | 100 | Min | 0.024 |
| | 10 | 100 | | |
| | 15 | 100 | | |
| $C_{6:0}$ (mg/g) | 0 | 51 | | |
| | 6 | 60 | Max | 0.026 |
| | 10 | 70 | | |
| | 15 | 77 | | |
| $C_{8:0}$ (mg/g) | 0 | 59 | | |
| | 6 | 88 | Max | 0.033 |
| | 10 | 92 | | |
| | 15 | 98 | | |
| $C_{10:0}$ (mg/g) | 0 | 62 | | |
| | 6 | 85 | Max | 0.042 |
| | 10 | 98 | | |
| | 15 | 100 | | |
| $C_{12:0}$ (mg/g) | 0 | 59 | | |
| | 6 | 84 | Max | 0.043 |
| | 10 | 97 | | |
| | 15 | 100 | | |
| $C_{14:0}$ (mg/g) | 0 | 62 | | |
| | 6 | 84 | Max | 0.056 |
| | 10 | 95 | | |
| | 15 | 100 | | |
| $C_{16:0}$ (mg/g) | 0 | 61 | | |
| | 6 | 84 | Max | 0.065 |
| | 10 | 95 | | |
| | 15 | 100 | | |
| $C_{18:0}$ (mg/g) | 0 | 64 | | |
| | 6 | 66 | Max | 0.070 |
| | 10 | 87 | | |
| | 15 | 96 | | |
| $C_{18:1}$ (mg/g) | 0 | 81 | | |
| | 6 | 91 | Max | 0.070 |
| | 10 | 96 | | |
| | 15 | 99 | | |
| $C_{18:2}$ (mg/g) | 0 | 62 | | |
| | 6 | 82 | Max | 0.066 |
| | 10 | 93 | | |
| | 15 | 99 | | |
| $C_{18:3}$ (mg/g) | 0 | 80 | | |
| | 6 | 88 | Max | 0.000 |
| | 10 | 92 | | |
| | 15 | 97 | | |
| TFFA (mg/g) | 0 | 59 | | |
| | 6 | 83 | Max | 0.498 |
| | 10 | 96 | | |
| | 15 | 100 | | |
| Inner pH | 0 | 100 | | |
| | 6 | 95 | Max | 6.660 |
| | 10 | 63 | | |
| | 15 | 30 | | |
| Lactose (mg/g) | 0–15 | 100 or 0 | Min | 35.730 |

[a] Storage temperature ($x_2$) was pre-fixed at 4°C, storage time ($x_1$) was pre-fixed at each experimental storage time and flushing gas composition ($x_3$) was left unconstrained in the whole experimental range.

[b] TO, type of optimum that best reproduces the ideal conditions for storage of *Requeijão* throughout time.

Table 4. It is interesting to note that the minimization of release of every FFA at 4°C as storage time elapses requires an increasing content of $CO_2$ in the flushing gas, a realization that is consistent with the known fact that $CO_2$ reduces microbial growth and, consequently, microbial lipolysis. All linear and quadratic effects encompassing all processing variables were important in what concerns lactose content of whey cheese (see Table 2), except the linear effect of flushing gas composition; the only negligible interaction was storage time/storage temperature. Hence, increasing storage temperature or storage time increases the lactose metabolism (as expected), but the composition of the flushing gas does not apparently play an important role. There was a true local minimum for this property (see Table 3); however, the main goal regarding lactose would be to minimize its degradation, i.e. to maximize its content in the final whey cheese. Hence, to obtain a minimum value for $\hat{y}$, a procedure similar to that used in the case of FFA was again adopted. The results depicted in Table 4 show that, at 4°C and under plain $CO_2$ or plain $N_2$, similar (and maximum) lactose contents are obtained. Therefore, the flushing gas composition plays a minor role in this respect.

All effects were important, except composition of the flushing gas via its linear effect and its interaction with storage temperature/overhead gas, regarding inner pH of whey cheese (see Table 2). In the case of outer pH, all linear and quadratic effects, as well as all interactions, of the processing variables were again important, except for flushing gas composition. The composition of the flushing gas did not play a significant role for lactose and lactic acid contents (see Table 3), which probably means that reduction of the outer pH is directly associated with lactose metabolism and, consequently, to lactic acid formation by microorganisms. No true global maximum exists for either the inner or the outer pH in whey cheese (see Table 3); however, it is important that the pH of this cheese does not decrease excessively during storage, so an actual maximum is sought for this parameter. In the case of the outer pH, there is a maximum with respect to one variable (flushing gas composition); hence, the maximum value for $\hat{y}$ (close to the initial value of pH) was based on the equation encompassing $x_3$ (see Table 3), with temperature pre-fixed at 4°C and storage time selected as unconstrained independent variable (with results plotted in Fig. 1a). The maximum value of the outer pH, closer to its initial value (6.17), is then obtained when $x_1 = 8.2$ days, $x_2 = 4$°C and $x_3 = 100$ % (v/v) $CO_2$. In the case of the inner pH, the only maximum found (for flushing gas) violates the experimental range utilized, so the problem was solved as described above for the lactose and FFA contents; results thereby obtained are depicted in Table 4. Inspection of this table indicates that, in order to maintain a value for the inner pH close to that of the reference whey cheese at 4°C, increases of the content of $N_2$

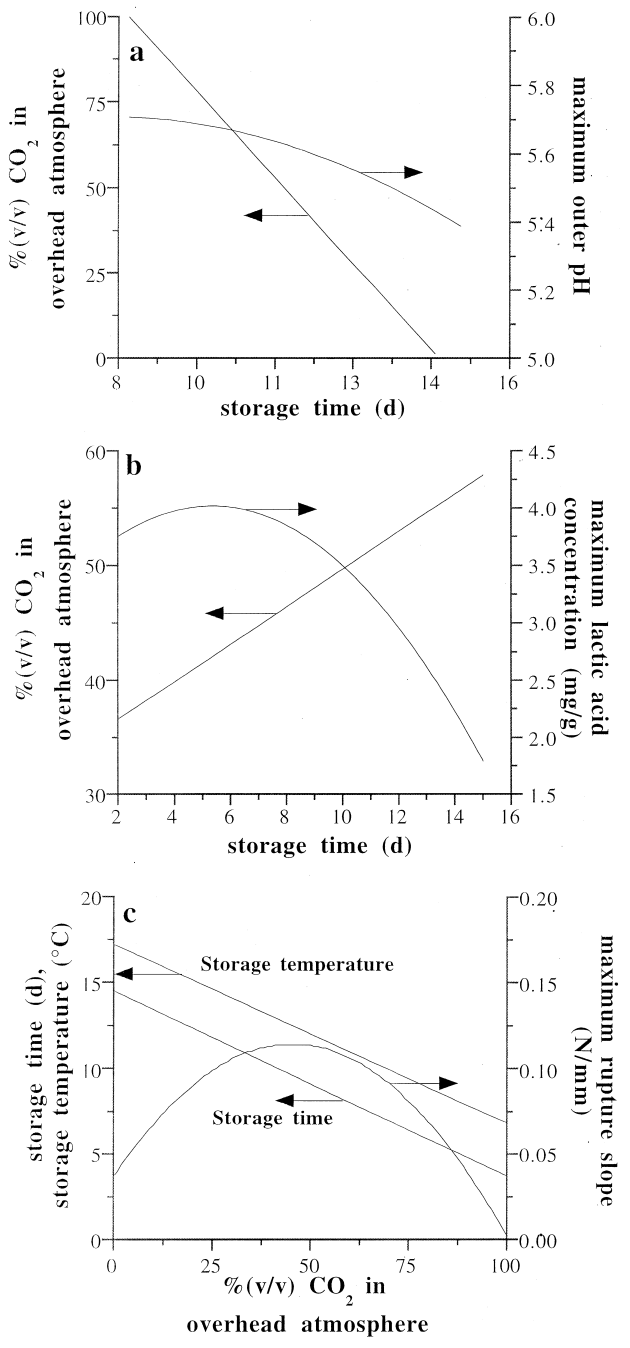


Fig. 1. Theoretical evolution of (a) outer pH and corresponding loci of the manipulated processing variables, based on the equation encompassing flushing gas composition, with temperature pre-fixed at 4°C and storage time selected as unconstrained independent variable in the interval 2–15 days; (b) lactic acid content and corresponding loci of the manipulated processing variables, based on the equation encompassing flushing gas composition, with temperature pre-fixed at 4°C and storage time selected as unconstrained independent variable in the interval 2–15 days; and (c) rupture slope and corresponding loci of the manipulated processing variables, based on the equations encompassing time and temperature, with overhead gas composition selected as unconstrained independent variable in the interval 0–100 % (v/v) $N_2$.

in the flushing gas composition are necessary as storage time elapses.

All quadratic effects were statistically significant, at the 5% level, regarding the content of lactic acid, as happened with the lactose content (see Table 2). In a similar manner, all linear effects were important (and positive), except for the composition of the flushing gas, and all interactions were also important, except storage temperature/flushing gas composition. A true local optimum could not be found once again (see Table 3); since the objective function was to minimize the amount of lactic acid formed, setting the storage temperature to 4°C via use of the equation encompassing $x_3$ (see Table 3) and setting the storage time as unconstrained independent variable within the experimental limits (see Fig 1b) leads to a minimum for the lactic acid content, that is closest to the reference (4.95 mg/g) when $x_1 =$ 5.4 days, $x_2 =$ 4°C and $x_3 =$ 42 % (v/v) $CO_2$.

The rupture slope is significantly affected (at the 5% level) by all interactions, but only slightly affected by storage time (via its quadratic effect) and by storage temperature (via its linear effect) (see Table 2). This means that only the combination of these factors affects the rigidity of the whey cheese, although in practice virtually no changes were observed in the experimental cheeses. A true local optimum could not be found (see Table 3), but two variables (storage time and storage temperature) permitted a minimum to be attained (since a lower slope is associated with lower rigidity, it is important to minimize this value); that minimum, which can be obtained if equations encompassing $x_1$ and $x_2$ (see Table 3) were pre-fixed and $x_3$ were selected as unconstrained independent variable, produces the curve in Fig. 1c. Inspection of this figure confirms that no true minimum can be attained. It is notorious that two minima, both within experimental limits, viz. $x_1 =$ 3.7 days, $x_2 =$ 7.0°C and $x_3 =$ 100 % (v/v) $CO_2$, on the one hand, and $x_1 =$ 12.6 days, $x_2 =$ 17.2°C and $x_3 =$ 100 % (v/v) $N_2$, on the other, can be reached; however, both minima obtained for the rupture slope are far from that for the reference cheese (0.097 N/mm), so the conditions that originate a value for the slope closer to that of the reference whey cheese are $x_1 =$ 7.2 days, $x_2 =$ 10.3°C and $x_3 =$ 68 % (v/v) $CO_2$.

Moisture content was not significantly affected by any of the variables tested, which was expected as all whey cheeses were packaged in high barrier bags that essentially prevent loss of water. This is an important advantage for packaging if extension of the shelf-life of this type of cheese were sought, as the unpackaged whey cheese dries out rapidly and thus loses its fresh appearance. No true local optimum could be found (see Table 3) for this parameter; in particular, no maximum was determined because the variables tested affect this parameter only to a very low extent.

### 3.4. Sensorial assessment

In the performance of the organoleptic tests, a whey cheese stored under regular storage conditions (i.e. stored unpackaged at 4°C under a stagnant atmosphere) was included as control against which all experimental whey cheeses were compared. The control was obviously included in each sensorial analysis, and was aimed at determining whether the organoleptic evaluation was impartial and consistent. From the data depicted in Table 5, it can be seen that the control at 2, 6 and 15 days scored 5.0, 5.2 and 4.7, respectively, for acidity, 5.3, 4.9 and 5.0, respectively, for brightness, and 4.9, 4.6 and 5.0, respectively, for overall visual aspect; by 10 days the replicated controls scored 5.9 and 5.1, 6.6 and 6.7, and 7.5 and 7.3 for acidity, brightness and overall visual aspect, respectively. Therefore, the sensorial evaluation could be qualified as good because these controls are very close to one another; it could also be concluded that blocking was not required at all for acceptable accuracy of the organoleptic evaluation. When one compares the acidity results of the experimental whey cheeses with those of the unpackaged whey cheese (control), it is obvious that the cheeses at earlier stages (2 and 6 days), at high storage temperature (18°C), and especially when plain nitrogen was employed, are more acidic than the control, an observation that confirms the results obtained for the outer pH in these whey cheeses. By 10 days, only the whey cheeses stored at 4°C were less acidic than the control, which also bore to a higher outer pH; by 15 days only the whey cheeses stored at 4°C were significantly less acid that the control. It is important to note that the significant difference observed in the outer pH between unpackaged and packaged *Requeijão* stored at 4°C under plain $CO_2$ was not associated with an equivalent difference in terms of sensorial analysis; this suggests that it is hard to assess accurately such a sensory feature, especially when other off-flavors can be simultaneously released; after 6 days the differences became more notorious. By 2 days, only the cheese stored at the highest temperature was brighter than the control cheese, probably because higher temperatures lead to faster release of water; however, after 2 days all experimental whey cheeses exhibited a brighter surface when compared with the control cheese, which supports once again the advantages of packaging. In terms of overall visual aspect by 2 days, the control cheese was the least preferred from the whole array of whey cheeses, which allows one to conclude that all types of package might increase the shelf life of whey cheese in terms of visual appearance. Although no taste analyses were performed, it was noteworthy that only the whey cheeses stored at 4°C could be distinguished from the control via a less acidic smell.

Statistical analyses of the results pertaining to acidity, brightness and overall appearance (see Table 6) were carried out with all samples, including the control. For acidity, a significant *F*-ratio was found (i.e. there are differences among the firmness of the samples), at the 1% level of significance, only for 2 and 6 days; for brightness, a significant *F*-ratio was found, at the same level of significance, for 6 and 15 days; for overall appearance, a significant *F*-ratio was found for 6 and 15 days. An additional experiment of packaging with air led to so poor results that it was dropped out from further consideration; such results included much worse performance than that of the stagnant, open air experiment, which means that the microbial factor is more

Table 5
Average acidity, brightness and overall appearance, obtained from a multiple comparison test, for the assessment of whey cheeses, packaged under different conditions, throughout storage using a whey cheese stored unpackaged at 4°C as reference

| Package conditions | Acidity[a] | Brightness[b] | Overall visual preference[c] |
|---|---|---|---|
| *2 days* | | | |
| 100% $CO_2$ — 4°C | 5.1c[d] | 5.5c | 5.2b |
| 100% $N_2$ — 4°C | 5.0c | 5.3c | 5.2b |
| 100% $CO_2$ — 18°C | 6.1b | 6.5ab | 6.4a |
| 100% $N_2$ — 18°C | 7.1a | 6.4ab | 5.6ab |
| 50% $CO_2$ — 12°C | 5.4bc | 5.7c | 5.9ab |
| Control | 5.0c | 5.3c | 4.9b |
| *6 days* | | | |
| 100% $CO_2$ — 4°C | 5.2c | 7.2c | 6.9c |
| 100% $N_2$ — 4°C | 4.6c | 7.3c | 7.1c |
| 100% $CO_2$ — 18°C | 6.4b | 6.6b | 6.7ab |
| 100% $N_2$ — 18°C | 8.1a | 7.2bc | 5.6c |
| 50% $CO_2$ — 12°C | 5.5bc | 7.1bc | 6.5bc |
| Control | 5.2c | 4.9ab | 4.6ab |
| *10 days* | | | |
| 100% $CO_2$ — 12°C | 4.9ab | 7.3b | 7.7ab |
| 100% $N_2$ — 12°C | 5.6b | 7.6ab | 7.7b |
| 50% $CO_2$ — 4°C | 4.0a | 6.9b | 7.5a |
| 50% $CO_2$ — 12°C[e] | 5.9b | 6.6a | 7.5b |
| 50% $CO_2$ — 18°C | 5.5b | 6.9b | 7.5b |
| Control | 5.1ab | 6.7ab | 7.3ab |
| *15 days* | | | |
| 100% $CO_2$ — 4°C | 3.9b | 7.1b | 7.8b |
| 100% $N_2$ — 4°C | 4.1b | 7.1b | 7.7b |
| 100% $CO_2$ — 18°C | 4.6b | 7.2b | 7.8b |
| 100% $N_2$ — 18°C | 6.4a | 7.3b | 7.6a |
| 50% $CO_2$ — 12°C | 4.4b | 7.2b | 7.8b |
| Control | 4.7b | 5.0a | 5.0b |

[a] Scale 1–9: 1, extremely less acidic than reference, 5, as acidic as reference, 9, extremely more acidic than reference.

[b] Scale 1–9: 1, extremely less bright than reference, 5, as bright as reference, 9, extremely brighter than reference.

[c] Scale 1–9: 1, with extremely worse aspect than reference, 5, with as good aspect as reference, 9, with extremely better aspect than reference.

[d] Two means with the same letter are not significantly different from one another ($P < 0.05$).

[e] The replicate corresponds to 50% (v/v) $CO_2$ — 12°C, whereas the others correspond to the reference cheese.

Table 6
ANOVA table for organoleptic data expressed as acidity, brightness and overall appearance

| Source of variation | Storage time | Number of degrees of freedom | Sum of squares | Mean square | *F*-ratio |
|---|---|---|---|---|---|
| *Acidity* | | | | | |
| Samples | 2 | 5 | 64.57 | 12.91 | 10.20[a] |
| | 6 | 5 | 149.75 | 29.95 | 20.93[a] |
| | 10 | 5 | 43.44 | 8.69 | 0.71 |
| | 15 | 5 | 73.73 | 14.75 | 1.81 |
| Panelists | 2 | 18 | 22.79 | 1.27 | |
| | 6 | 18 | 25.75 | 1.43 | |
| | 10 | 18 | 220.49 | 12.25 | |
| | 15 | 18 | 146.82 | 8.16 | |
| Sensory test error | 2 | 90 | 86.26 | 0.96 | |
| | 6 | 90 | 122.25 | 1.36 | |
| | 10 | 90 | 152.56 | 1.70 | |
| | 15 | 90 | 152.44 | 1.69 | |
| *Brightness* | | | | | |
| Samples | 2 | 5 | 27.10 | 5.42 | 2.50[b] |
| | 6 | 5 | 75.96 | 15.19 | 7.77[a] |
| | 10 | 5 | 10.25 | 2.05 | 0.76 |
| | 15 | 5 | 73.47 | 14.69 | 3.47[a] |
| Panelists | 2 | 18 | 39.02 | 2.17 | |
| | 6 | 18 | 35.19 | 1.96 | |
| | 10 | 18 | 48.74 | 2.71 | |
| | 15 | 18 | 76.28 | 4.24 | |
| Sensory test error | 2 | 90 | 53.40 | 0.59 | |
| | 6 | 90 | 32.70 | 0.36 | |
| | 10 | 90 | 41.58 | 0.46 | |
| | 15 | 90 | 31.19 | 0.35 | |
| *Overall appearance* | | | | | |
| Samples | 2 | 5 | 28.32 | 5.66 | 2.21 |
| | 6 | 5 | 89.34 | 17.87 | 4.48[a] |
| | 10 | 5 | 1.89 | 0.38 | 0.25 |
| | 15 | 5 | 119.87 | 23.97 | 11.79[a] |
| Panelists | 2 | 18 | 46.09 | 2.56 | |
| | 6 | 18 | 71.77 | 3.99 | |
| | 10 | 18 | 27.42 | 1.52 | |
| | 15 | 18 | 36.61 | 2.03 | |
| Sensory test error | 2 | 90 | 132.02 | 206.42 | |
| | 6 | 90 | 115.49 | 276.61 | |
| | 10 | 90 | 21.11 | 50.42 | |
| | 15 | 90 | 20.96 | 177.45 | |

[a] Significant at the 1% level (critical value of $F$ is 3.47).
[b] Significant at the 5% level (critical value of $F$ is 2.30).

important than the driness factor (which plays a role in open air experiments).

## 4. Conclusions

Storage of whey cheeses above refrigeration temperatures led to important decreases of both the inner and outer pH values for all flushing gas compositions tested; however, rupture slope and moisture content did not undergo major variations. Lactose and total FFA content did not exhibit relevant variations at 4°C for any of the flushing gas compositions, whereas lactic acid exhibited a slight decrease. Minimization of release of every FFA at 4°C as storage time elapses requires increases in the content of $CO_2$ in the flushing gas, hence proving that plain $CO_2$ inhibits completely lipolysis at all storage temperatures experimented. Increasing storage temperature or storage time increases the rate and extent of lactose metabolism, respectively, but the composition of the initial flushing gas did not play an important role toward lactose and lactic acid contents, as did in the case of the inner pH. Global maxima or minima were found for some parameters; however, all optima lie on experimental constrains, so restrictions had to be imposed on each variable in order to produce reasonable processing optima.

Assessment of overall visual appearance indicated that all types of package can increase the shelf life of whey cheese; however, preference in terms of acidic smell was only confirmed for whey cheeses stored at 4°C.

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## References

Balcão, V. M., & Malcata, F. X. (1998). Interesterification and acidolysis of butterfat with oleic acid by *Mucor javanicus* lipase: changes in the pool of fatty acids residues. *Enzyme and Microbial Technology, 22*, 511–519.

Berne, S. (1994). MAP-ing the future with CAP-ability. *Prepared Foods*, March, 101–105.

Box, G. E. P., Hunter, W. G., & Hunter, J. S. (1978). *Statistics for experiments — an introduction to design, data analysis and model building*. New York: Wiley (pp. 510–535).

Brennan, J. C., Jowitt, R., & Williams, A. (1975). An analysis of the action of the general foods texturometer. *Journal of Texture Studies, 6*, 83–100.

Cavaliere, A. (1988). Characteristics of some dairy products produced in the Diano Valley from ewe's milk. *Mondo de Latte, 42*, 599–604.

Fedio, W. M., Macleod, A., & Ozimek, L. (1994). The effect of modified atmosphere packaging on the growth of microorganisms in cottage cheese. *Milchwissenschaft, 49*, 622–628.

Fedio, W. M., Ozimek, L., & Wolfe, F. H. (1994). Gas producing during storage of Swiss cheese. *Milchwissenschaft, 49*, 3–7.

Ferraz, A. (1998). Adaptação ambiental no sector dos lacticínios. *AIP Ambiente, 22*, 22–29.

Garcia, H. S., Reyes, H. R., Malcata, F. X., Hill, C. G., & Amundson, C. H. (1990). Determination of the major free fatty acids in milkfat using a three component mobile phase for HPLC analysis. *Milchwissenshaft, 45*, 757–759.

Hotchkiss, J. H. (1988). Experimental approaches to determining the safety of food packaged in modified atmospheres. *Food Technolology, 42*, 55–64.

IDF (1958). *Determination of dry matter in cheese and processed cheese: Standard 4*. Brussels: International Dairy Federation.

Mathews, M. E. (1984). Whey protein recovery processes and products. *Journal of Dairy Science, 67*, 2680–2692.

Mathur, B. N., & Shahani, K. M. (1981). Ricotta cheese could be your best vehicle for whey. *Dairy Field, 164*, 110–114.

Meilgaard, M., Civille, G. V., & Carr, B. T. (1988). *Sensory evaluation techniques*. Boca Raton: CRC (pp. 47–111).

Modler, H. W., & Emmons, D. B. (1989). Production and yield of whole-milk Ricotta manufacture by a continuous process. II. Results and discussion. *Milchwissenschaft, 44*, 753–756.

Moir, C. J., Eyles, M. J., & Davery, J. A. (1993). Inhibition of pseudomonas in cottage cheese by packaging in atmospheres containing carbon dioxide. *Food Microbiology, 10*, 345–351.

Mulvihill, D. M. (1991). Trends in the production and utilization of dairy protein products: production. *Food Research Quarterly, 51*, 145–157.

Nunez, M., Garcia-Aser, C., Rodriguez-Martin, M. A., Medina, M., & Gaya, P. (1986). The effect of ripening and cooking temperature on proteolysis and lipolysis in Manchego cheese. *Food Chemistry, 21*, 115–123.

Pereira, A. J. G., Póvoa, M. E. B., & Cruz, G. R. (1982). Influência da velocidade de aquecimento sobre a qualidade do Ricotta. In *VII congresso nacional de lacticínios,* Juiz de Fora, Brazil.

Pintado, M. E., Lopes da Silva, J. A., & Malcata, F. X. (1996). Characterization of *Requeijão* and technological optimization of its manufacturing process. *Journal of Food Engineering, 30*, 363–376.

Pintado, M. E. and Malcata, F. X. (2000). Characterization of whey cheese packed under vacuum. *Journal of Food Protection. 63*, 216–221.

Roseiro, M. L. B., & Wilbey, R. A. (1991). The effect of whey storage on the production of whey cheese. *Sheep and Dairy News, 3*, 25–44.

Rosenthal, I., Rosen, B., Bernstein, S., & Popel, G. (1991). Preservation of fresh cheeses in a $CO_2$-enriched atmosphere. *Milchwissenschaft, 46*, 706–708.

Santiago, L. (1993). O *Requeijão* em Portugal. *Via Láctea, 2*, 71–73.

Vodret, A. (1970). La ricotta pecorina sarda. *Scienza et Tecnica Lattiero-Casearia, 21*, 310–313.

Weatherup, W. (1986). The effect of processing variables on the yield and quality of Ricotta cheese. *Dairy Industry International, 51*, 41–45.

Wingerd, W. H., Saperstein, S., & Lutwak, L. (1970). Bland, soluble, whey protein concentrate has excellent nutritional properties. *Food Technology, 24*, 758, 760–761, 764.

Zall, R. R. (1984). Trends in whey fractionation and utilization, a global prespective. *Journal of Dairy Science, 67*, 2621–2629.

Ziino, M., Salvo, F., Stagno-d'Alcontres, I., & Chiofalo, B. (1993). Composition of Ricotta cheese from Pinzirita sheep bred in the areas surrounding Mt. Etna volcano (Catania–Italy). *Scienza et Tecnica Lattiero-Casearia, 44*, 217–231.

Zoon, P. (1991). The relation between instrumental and sensory evaluation of the rheological and fracture properties of cheese. *Bulletin of the International Dairy Federation, 268*, 30–35.