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Extending the shelf-life and proximate composition stability of ready to eat foods in vacuum or modified atmosphere packaging

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Abstract

The stability of the proximate composition (moisture, proteins, lipids and ash) and the microbiological state of cooked 'ready to eat' foods in vacuum or modified atmosphere packaging were examined and compared with conventionally packaged (in air) foods. The study was carried out for 7 and 29 days, during which time the food products were stored at 3°C. Vacuum or modified atmosphere packaging were effective for prolonging the shelf-life of the studied products up to 29 days with minimal changes in the proximate composition. Aerobic mesophilic, psychrotrophic microrganisms, and yeasts and moulds increased with time regardless of packaging type but more rapidly under conventional packaging.

Keywords: Shelf-life; Packaging; Proximate composition

1. Introduction

Consumer markets are showing an increased demand for a new class of processed foods, the ready to eat products (M.A.P.A., 1999). Cooked ready to eat products are most often consumed without further cooking, and therefore, the presence of pathogens presents a considerable food safety threat (Muriana et al., 2002). The catering business, including restaurants and canteens (schools, hospitals, nursing homes, prisons, etc.) has developed systems for the preparation and preservation of different items which can be served at the opportune moment without any reduction in the final quality (Austin et al., 1998; Hao et al., 1998).

Changes are occurring in the home, too. More women go out to work and there is increasing pressure on people to work harder and longer, leaving less time for daily shopping. Furthermore, the concept of eating a healthy and balanced diet is declining; hence, the need for convenience foods that taste, smell, and feel like home-made foods (Katz, 1999).

Such foods would preferably be modelled on traditional foods, reduce preparation and planning time, provide a pleasurable sensory experience and involve little effort on the part of the cook (Sloan, 2000).

The addition of chemical additives (preservatives, antioxidants, colorants, etc.) has frequently been associated with certain health problems, including allergies, and other more serious illnesses such as the initiation of carcinogenesis (Halliwell et al., 1995). In light of this, there is a growing tendency for consumers to shun chemically treated products in favour of more natural products, a trend which lends weight to the advantages of the so-called Mediterranean diet (Martínez-Tomé et al., 2001). Faced with this demand, there is a need to develop techniques to maintain the natural qualities of cooked ready to eat foods without using chemical preservatives by using, for example, vacuum packaging or modified atmosphere packaging (MAP) (Day, 1998).

All this requires a combination of raw product quality, special attention to the production chain, careful handling and the strict control of distribution temperatures (Coventry et al., 1995; Bharti and Sahoo, 1999; Brody, 2000). Foods must be prepared at relatively high temperatures (around 100°C) and then packed in vacuum or modified atmosphere. The process is rounded off by a rapid cooling step followed by refrigerated storage. The end product should be reheated at approximately 70°C for 2 min before consumption (Sallarés, 1995).

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MAP costs twice as much as vacuum packaging because it requires special packaging material and gases (Reddy et al., 1992). The elimination of O_2 from the packaging and the introduction of different concentrations of CO_2 and N_2, together with adequate refrigeration, inhibits the growth of aerobic micro-organisms, proteolytic bacteria, yeasts and fungi (Swiderski et al., 1997). However, Francis and O'Beirne (1998) suggested that the gas atmospheres present within packages of vegetables may increase the growth of *Listeria*, possibly by altering the levels of general competitive microflora since certain micro-organisms can grow at refrigeration temperatures; it is precisely these micro-organisms which are the most worrying from a microbiological safety point of view. In this respect, five pathogenic bacteria are known to grow on food below 5°C (*Clostridium botulinum*, *Listeria monocytogenes*, *Yersinia enterocolitica*, enterotoxigenic *Escherichia coli* and *Aeromonas hydrophila*) and another five at temperatures just above 5°C (*Staphylococcus aureus*, *Clostridium perfringens*, *Salmonella* spp., *Vibrio parahaemolyticus* and *Bacillus cereus*) (Devlieghere et al., 2000; Farber, 1991). Harrison et al. (2000) concluded that refrigerated modified atmosphere packaged foods should be maintained at <4°C to ensure product safety with respect to *Y. enterocolitica*.

Vacuum and MAP have certain advantages over other more traditional methods since such practices increase the shelf-life of raw vegetables by 50–400% by reducing respiration and ethylene production, delaying ripening and softening and reducing chlorophyll degradation, while at the same time maintaining organoleptic characteristics (taste, aroma, texture) (Francis et al., 1999).

The ability of MAP to extend shelf-life has been studied by many authors and some excellent papers have been published on, for example, cod (Guldager et al., 1998), channel cat fish (Silva and White, 1994), cooked turkey (Juneja et al., 1996), chicken (Young et al., 1989), potato stew and meat in sauce (Goto et al., 1995).

Spain plays an important role in the European market for modified atmosphere products not so much as a consumer but as producer of meat, fruit and vegetables (Marketpower, 1988). Demand for MAP products in UK and France grew by about 40% and 25%, respectively, between 1994 and 1999 (MSI, 1998).

Few studies have been conducted on the composition parameters and microbiological quality of cooked traditional dishes prepared as ready to eat products using vacuum or MAP. Our aim was to investigate the microbiological spoilage indicators and proximate composition of cooked ready to eat foods preserved in different types of industrial packaging (vacuum or modified atmosphere) without chemical additives and to compare the results with those obtained with conventional packaging.

2. Materials and methods

The different cooked 'ready to eat' foods were prepared by Elior-Osesa-Catering Casa Tomás, Murcia (Spain) in a traditional manner from several fresh high-quality ingredients, using a 25 m^2 cooker with all the necessary accessories, and under laminar flow (Telstar® S.A.); the sterile air (4000 m^3/h) was obtained with HEPA filters of 0.3 micro particle size and 99.97% efficiency.

Strict hygiene and quality control conditions were followed during food preparation. Approximately 250 g of cooked food were placed in a packaging CPET (crystalline polyester) (Tecnopack®) and immediately refrigerated with an AREX® model HC 202-250 rapid cooler (Italy) to an internal temperature of 3°C in half an hour or less (FDA, 1993). Such fast cooling of foods is critical. The food was then covered with a single layer of polyester film (Tecnopack®). The packaging conditions used were (a) conventional (packed in air and sealed with no vacuum or gases), (b) vacuum (99%) (c) modified atmospheres of carbon dioxide and nitrogen (80/20, CO_2/N_2) (Carburos Metálicos, Madrid, Spain) using an ILPRA® model FP BASIC VG (Italy). Containers containing no food were sealed in identical conditions to act as packaging control. In all cases, samples of the prepared foods were taken in sterile packs to detect possible contamination by micro-organism at the time of packaging. A Qualipack® 170 (Meggitt Controls, England) was used for sealing. The gas composition of the different packages was measured by gas analyser (Abis Print Horse Power s.r.l., Milano, Italy). For samples flushed with CO_2 and N_2, the oxygen content was verified to be less than 1% using a Mocon® electronic oxygen analyser.

HACCP was previously implemented according to the legislation established by EC Directive (1993) and a questionnaire was used for foodhandler training as a useful tool for improving GMP during preparation (Martínez-Tomé et al., 2000). The samples were sent to our laboratory in refrigerated conditions.

2.1. Experimental design

This study comprised in three parts:

1. Lentil soup, meat stew and meat, legume and vegetable soup stored for 7 days at 3°C in different packagings (conventional, vacuum and modified atmosphere of 80% CO_2 and 20% N_2).
2. The same products in the same conditions but stored for 29 days.
3. Other cooked foods (see Table 5) packed in modified atmosphere conditions (80% CO_2 and 20% N_2) and stored for 29 days at 3°C.

At once a week, an appropriate aliquot was taken from three packs of each treatment for microbiological and proximate composition analysis.

2.2. Microbiological analysis

Samples (25 g) were blended in buffered peptone water (BPW) (225 ml), homogenized using a Stomacher (IUL Masticator No.0927/94 type 0400) (Harrison et al., 1996) and serially diluted.

Aerobic plate counts (APC, 37°C, 24 h) were determined by surface spreading homogenate dilutions (0.1 ml) on Plate Count Agar (Merck Laboratories, Darmstadt, Germany) (Prokopowich and Blank, 1991). To isolate *S. aureus*, a 0.1 ml sample was spread on Baird-Parker agar plates (BPA, Merck) (Prokopowich and Blank, 1991) and incubated at 37°C for 24–48 h. Typical black colonies (gram-positive, catalase-positive cocci) were subjected to the tube coagulase test (Difco Laboratories, Detroit, Michigan, USA).

For mould and yeast detection, 0.1 ml of sample was spread on Oxytetracycline Glucose Yeast agar base (Merck) and incubated at ambient temperature (22°C) for 5 days (Mossel et al., 1970).

Psychrotrophic populations in the samples were determined by surface spreading of the serial dilutions on Plate Count Agar, which were then incubated aerobically at 5°C for 7–10 days. A selective medium for such populations was not used (Sheridan, 1995).

To isolate *Lancefield group* D Streptococci, food samples were spread on Kanamycin Aesculin Azide agar (KAA, Merck) plates and incubated at 37°C for 24 h.

Anaerobic populations were determined by tube dilution on SPS agar (Perfringens Selective Agar, according to Angelotti et al. (1962), Merck) and TSN agar (Perfringens Selective Agar according to Marshall et al. (1965), (Merck) before being incubated in anaerobic jars using CO_2 generator packs (Anaerocult A Merck) at 46°C for 24 h. Colony forming units were then counted (Harrison et al., 1996).

Total *Enterobacteriaceae* (37°C, 18–24 h) were determined by surface spreading of homogenized dilutions (0.1 ml) on McConkey Agar (Merck). Total coliforms were determined by the most probable number procedure using lauryl sulphate tryptose broth (LTB, Merck) followed by confirmation of gas-positive tubes using Brilliant Green Lactose Bile Broth (BGBB, Merck). Incubation in both cases was at 37°C for 24–48 h (Prokopowich and Blank, 1991). Faecal coliforms (*E. coli*) were determined using BGBB incubated at 44.5°C for 24–48 h.

To detect *Salmonella*, 1.0 ml of a solution of BPW sample incubated for 24 h was added to 10 ml of Selenite Enrichment Broth (Merck). In addition, a 10 ml peptone water sample was added to Tetrathionate Broth Base, according to Muller-Kauffmann (100 ml, Merck), and both incubated at 37°C and 42°C, respectively for 24 h. Subcultures were then made onto Brilliant-green Phenol-red Lactose Sucrose agar plates (BPLS, Merck) and *Salmonella–Shigella* agar (SS agar, Merck) by streaking and incubating overnight at 37°C (Tietjen and Fung, 1995). *Salmonella–Shigella* suspects were tested with biochemical tests (API 20E, bioMerieux s.a., Mercy L'Etoile, France).

For the detection of *Listeria monocytogenes*, 25 g samples were blended in Fraser I (225 ml, Merck), homogenized by using a Stomacher and incubated at 37°C for 18–24 h (first enrichment). Subcultures were made onto Oxford Agar (Merck), inoculating 0.1 ml and incubating at 37°C for 48 h. After the first enrichment, 1.0 ml of Fraser I was added to 10 ml Fraser II (Merck) and incubated at 37°C for 18–24 h (second enrichment). Subsequently, 0.1 ml of Fraser II was spread on Oxford Agar and incubated at 37°C for 48 h (Fraser and Sperber, 1988).

2.3. Proximate composition analysis

Samples were lyophilized in an FTSSYSTEMS™ (GIRALT®) freeze-drier and the moisture content was then determined (Murcia et al., 1999). The freeze-dried samples were pulverized to a fine powder with a Moulinex model 505.

2.3.1. Ash and protein content

Ash was determined according to an AOAC (1990) method. For protein determination, we followed the AOAC method using a Carlo Erba model AE 1108 elemental analyser with a Porapack QS (25 cm) GC column and a Thermic Conductivity Detector. Standard sample materials (EDTA, nicotinic acid, tryptophan and lysine, HCl) were place in a tared standard tin capsule for calibration and performance testing. Helium at 1.94×10^{-3} l/s was used as carrier gas; the reactor temperature was 1020°C. The chromatographic oven temperature was 65°C and the filament temperature 190°C. Samples weighing 1 mg were processed in these conditions, using V_2O_5, WO_3 and MgO as additives. The range was from 0.01% to 100%, with a standard deviation of 0.001%. The results of the nitrogen content were multiplied by 6.25 to obtain the protein percentage (Bellomonte et al., 1987).

2.3.2. Analysis of lipids

The gravimetric determination of the total lipid content was carried out according to the method described by Bligh and Dyer (1959).

The certified reference samples for control analysis were Standard Reference Material (apple leaf powder SRM 1515) from the National Institute of Standard and Technology (NIST).

2.4. Data analysis

The data were analysed using the Statistical Package for Social Sciences Windows 9.0. Analysis of variance (ANOVA) was carried out after triplicate experiments, calculating the significance level ($P<0.05$) by using the LSD multiple range test.

3. Results and discussion

This study investigated the prolongation of the shelf-life of cooked ready to eat foods elaborated in a traditional manner and stored in different packaging conditions. For this, microbial tests were carried out during a very short refrigerated storage period (7 days) in the case of cooked food (lentil soup, meat stew, meat–legume and vegetable soup). Only the data for aerobic mesophilic, psychrotrophic and yeasts and moulds are shown in Table 1. The results indicate that only the conventionally stored samples began to show low microbial counts in the samples analysed by the third day. The Enterobacteriaceae, *St. aureus*, *Streptococcus* D, anaerobic, total coliforms, *E. coli* and *Salmonella–Shigella* counts were negative for all the different types of industrial packaging used. The samples packed in vacuum or modified atmosphere showed significant differences from these conventionally packed samples because no bacterial growth (i.e. <10 cfu/g) was observed during the 7 days, pointing to the advantage of both processes in extending shelf-life (Table 1).

The most likely explanation of the differences observed in the microbiological results was the oxygen content, since aerobic conditions would have facilitated rapid microbial proliferation and it is known that gas packaging can greatly influence microbial activity (Huss, 1988).

Table 2 shows the results obtained for the proximate composition (moisture, protein, fat and ash) of lentil soup stored in different packaging conditions (conventional, vacuum, modified atmosphere packaging) for up to 7 days at 3°C. Samples stored in air had a slightly

Table 1
Microbiological analysis of cooked food samples stored in different packaging types for 7 days at 3°C[a]

Counts (log cfu/g)[b]	Food sample	Packaging	Days of storage at 3°C				
			Control[c]	1	3	5	7
Aerobic mesophilic	Lentil soup	Conventional	—[d]	—	—	2.12±0.16	2.08±0.13
		Vacuum	—	—	—	—	—
		MAP[e]	—	—	—	—	—
	Meat stew	Conventional	—	—	—	—	1.90±0.14
		Vacuum	—	—	—	—	—
		MAP	—	—	—	—	—
	Meat, legume and Vegetable soup	Conventional	—	—	—	1.90±0.13	2.00±0.12
		Vacuum	—	—	—	—	—
		MAP	—	—	—	—	—
Psychotrophic	Lentil soup	Conventional	—	—	1.00±0.01	2.16±0.20	2.20±0.21
		Vacuum	—	—	—	—	—
		MAP	—	—	—	—	—
	Meat stew	Conventional	—	—	—	—	2.00±0.11
		Vacuum	—	—	—	—	—
		MAP	—	—	—	—	—
	Meat, legume and vegetable soup	Conventional	—	—	2.02±0.11	2.00±0.11	2.10±0.14
		Vacuum	—	—	—	—	—
		MAP	—	—	—	—	—
Yeasts and moulds	Lentil soup	Conventional	—	—	—	—	—
		Vacuum	—	—	—	—	—
		MAP	—	—	—	—	—
	Meat stew	Conventional	—	—	—	—	—
		Vacuum	—	—	—	—	—
		MAP	—	—	—	—	—
	Meat, legume and vegetable soup	Conventional	—	—	—	—	—
		Vacuum	—	—	—	—	—
		MAP	—	—	—	—	—

[a] Data represent mean ± standard derivation of three replications.
[b] Enterobacteriaceae, *St. aureus*, *Streptococcus* D, anaerobic, total coliforms, *E. coli*, *Salmonella–Shigella* and *Listeria* resulted negative.
[c] Sample in sterile pack analysed on day 0.
[d] Not detected.
[e] MAP: Modified atmosphere packaging (80/20, CO_2/N_2).

Table 2
Moisture, protein, fat and ash content of lentil soup stored in different packaging conditions at 3°C for 7 days[a]

Assays (%)	Packaging	Days of storage at 3°C					
		1	2	3	4	5	7
Moisture	Conventional	83.00±1.50	83.30±1.51	83.80±1.51	83.20±1.48	83.40±1.45	—[b]
	Vacuum	82.30±1.49	81.20±1.47	82.30±1.49	81.80±1.46	81.70±1.45	81.50±1.45
	MAP[c]	81.50±1.45	81.60±1.46	81.80±1.46	81.70±1.42	82.00±1.48	82.20±1.49
Protein	Conventional	3.00±0.31	3.20±0.30	3.80±0.50	3.10±0.05	3.40±0.35	—
	Vacuum	3.20±0.31	3.90±0.37	3.60±0.51	3.80±0.51	3.30±0.34	3.50±0.40
	MAP	3.20±0.30	3.90±0.35	3.90±0.32	3.10±0.06	3.50±0.41	3.60±0.49
Fat	Conventional	1.30±0.06	1.30±0.07	1.10±0.05	1.10±0.03	1.40±0.32	—
	Vacuum	0.90±0.02	1.10±0.04	0.90±0.05	1.40±0.32	1.00±0.01	1.30±0.05
	MAP	1.40±0.30	1.50±0.34	1.40±0.29	1.20±0.20	1.30±0.07	1.30±0.10
Ash	Conventional	0.98±0.07	0.95±0.06	0.94±0.06	1.02±0.02	0.99±0.07	—
	Vacuum	1.03±0.03	1.00±0.01	0.97±0.05	0.99±0.08	0.98±0.06	0.97±0.04
	MAP	0.97±0.03	0.98±0.08	1.06±0.03	0.97±0.05	0.99±0.05	0.98±0.07

[a] Data represent mean±standard derivation of three replications.
[b] Not evaluated. Sensory scores (colour and flavour) unacceptable.
[c] MAP: Modified atmosphere packaging (80/20, CO_2/N_2).

Table 3
Microbiological analysis of cooked food stored in different packaging conditions for 29 days at 3°C[a]

Counts (log cfu/g)[b]	Food sample	Packaging	Days of storage at 3°C						
			1	3	7	10	14	21	29
Aerobic	Lentil soup	Conventional	—[c]	1.00±0.07	1.18±0.20	1.70±0.22	—	—	2.24±0.13
Mesophilic		Vacuum	—	—	—	—	—	1.00±0.06	1.70±0.20
		MAP[d]	—	—	—	—	—	—	—
	Meat stew	Conventional	—	—	—	2.00±0.16	2.00±0.15	2.00±0.11	2.60±0.23
		Vacuum	—	—	—	—	—	—	2.00±0.10
		MAP	—	—	—	—	—	—	2.30±0.20
	Meat, legume and vegetable Soup	Conventional	—	—	1.00±0.05	2.00±0.14	2.00±0.15	2.38±0.21	2.56±0.22
		Vacuum	—	—	—	—	—	—	—
		MAP	—	—	—	—	—	1.00±0.04	—
Psychotrophic	Lentil soup	Conventional	—	—	1.00±0.05	—	1.15±0.16	—	1.90±0.12
		Vacuum	—	—	—	—	1.00±0.06	—	—
		MAP	—	—	—	—	—	—	—
	Meat stew	Conventional	—	—	—	2.00±0.11	2.23±0.12	2.78±0.30	2.90±0.32
		Vacuum	—	—	—	—	—	—	1.48±0.25
		MAP	—	—	—	—	—	1.00±0.05	1.00±0.06
	Meat, legume and vegetable soup	Conventional	—	—	2.00±0.10	2.00±0.11	—	2.78±0.31	2.94±0.32
		Vacuum	—	—	—	—	—	—	2.14±0.15
		MAP	—	—	—	—	—	—	2.00±0.10
Yeasts and moulds	Lentil soup	Conventional	—	—	—	—	—	1.90±0.12	2.00±0.11
		Vacuum	—	—	—	—	—	—	—
		MAP	—	—	—	—	—	—	—
	Meat stew	Conventional	—	—	—	—	1.90±0.12	2.15±0.15	2.74±0.30
		Vacuum	—	—	—	—	—	—	1.78±0.22
		MAP	—	—	—	—	—	—	—
	Meat, legume and vegetable soup	Conventional	—	—	—	—	1.00±0.04	2.00±0.10	2.78±0.30
		Vacuum	—	—	—	—	—	—	1.70±0.21
		MAP	—	—	—	—	—	1.00±0.03	—

[a] Data represent mean ± standard derivation of three replications.
[b] Enterobacteriaceae, *St. aureus*, *Streptococcus* D, anaerobic, total coliforms, *E. coli*, *Salmonella–Shigella* and *Listeria* resulted negative.
[c] Not detected.
[d] MA: Modified atmosphere packaging (80/20, CO_2/N_2).

Table 4
Moisture, protein, fat and ash content of different cooked foods stored in modified atmosphere packaging (80/20, CO_2/N_2) at 3°C for 29 days[a]

Assays (%)	Control[b]	Days of storage at 3°C				
		MAP[c]				
	0	1	3	7	16	29
Lentil soup						
Moisture	82.60±1.40	80.90±1.30	82.10±1.50	82.00±1.30	80.80±1.10	80.60±1.20
Protein	3.80±0.51	3.20±0.30	3.80±0.50	3.00±0.26	4.00±0.45	4.10±0.46
Fat	2.10±0.16	1.90±0.14	2.00±0.14	2.10±0.16	1.80±0.12	1.70±0.10
Ash	1.30±0.20	0.90±0.02	1.35±0.03	1.24±0.03	1.33±0.04	0.94±0.02
Meat, legume and vegetable soup						
Moisture	68.60±1.00	67.30±1.10	66.80±1.30	68.00±1.00	67.80±1.30	66.60±1.30
Protein	8.80±0.07	8.20±0.02	9.10±0.05	8.70±0.03	9.00±0.06	8.50±0.01
Fat	5.20±0.50	4.90±0.10	4.50±0.10	5.00±0.40	4.60±0.20	4.40±0.10
Ash	1.36±0.07	1.36±0.06	1.42±0.11	1.56±0.15	1.33±0.04	1.40±0.10
Meat stew						
Moisture	78.60±1.50	77.30±1.30	76.30±1.40	76.50±1.50	76.80±1.10	76.60±1.10
Protein	9.80±0.03	9.70±0.08	9.40±0.06	9.20±0.04	9.30±0.01	9.20±0.05
Fat	8.20±0.40	7.90±0.20	8.30±0.30	7.90±0.20	8.10±0.60	8.00±0.30
Ash	1.05±0.21	0.93±0.19	0.98±0.20	0.93±0.17	0.99±0.21	0.70±0.02

[a] Data represent mean±standard derivation of three replications.
[b] A sample packaged in air was evaluated at "day 0" as control.
[c] MAP: Modified atmosphere packaging.

higher water content than vacuum or modified atmosphere packed samples. The moisture content ranged from 81.2% to 82.3% in vacuum and from 81.5% to 82.2% in MAP during the first 7 days although there were no significant differences ($P<0.05$) between the three treatments. The protein levels ranged from 3.0% to 3.9%, while the lipid content ranged between 0.9% and 1.5%. The ash content varied between 0.94% and 1.06%, with no significant differences ($P<0.05$) being observed between vacuum and modified packaging conditions.

The appearance of conventionally stored samples was unacceptable by the 7th day, and so these were not evaluated. Apparently, vacuum and modified atmosphere packaging offered an improvement as regards rancidity and warmed-over flavour (Nolan et al., 1989) with respect to packaging in air, which could be related with the low partial pressure of the oxygen in the package (Severini et al., 1998).

Our data are consistent with results from other studies, in which beef samples presented a slightly higher percentage of moisture in air packaging than in N_2/CO_2 MAP or vacuum packaging, while the fat content did not differ between treatments (Hwang et al., 1990).

Evaluation of the influence of vacuum or modified atmosphere packaging conditions compared with conventional packaging on the proximate composition of meat stew and meat–legume and vegetable soup stored for 7 days at 3°C (data not shown) showed similar results with no significant variations ($P<0.05$) to those shown in Table 2.

In a further study, microbiological counts were conducted when samples (lentil soup, meat stew and meat–legume and vegetable soup) were kept for 29 days at 3°C (Table 3). Samples stored in conventional air packaging had higher ($P<0.05$) microbial counts (aerobic mesophilic, psychrotrophic and yeast and moulds) than the corresponding samples kept in vacuum or modified packaging and growth was evident by day 3 or 7. In vacuum and modified atmospheres (80:20, CO_2:N_2) samples did not differ ($P<0.05$) from each other, and growth was only observed after day 14 for vacuum and day 21 for MAP onwards. No pathogens were recorded

Table 5
Microbiological analyses of cooked food stored in modified atmosphere packaging (80/20, CO_2/N_2) for 29 days

Food sample[a]	Aerobic mesophilic (log cfu/g)								Psychotrophic (log cfu/g)								Yeasts and moulds (log cfu/g)							
	Days of storage at 3ºC																							
	1	3	6	9	13	17	21	29	1	3	6	9	13	17	21	29	1	3	6	9	13	17	21	29
Spanish omelette	—[b]	—	—	—	—	—	2.57± 0.13	2.71± 0.15	—	—	—	—	—	—	2.71± 0.15	2.78± 0.16	—	—	—	—	—	—	2.36± 0.17	2.92± 0.25
Tuna and macaroni	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3.15 ± 0.30
Spaghetti and lean pork	—	—	—	—	—	—	—	2.04 ± 0.13	—	—	—	—	—	—	—	1.98 ± 0.12	—	—	—	—	—	—	1.50 ± 0.05	2.05 ± 0.12
Rice and meat	—	—	—	—	—	—	—	2.15± 0.25	—	—	—	—	—	—	2.00 ± 0.11	1.90 ± 0.11	—	—	—	—	—	—	—	2.35 ± 0.16
Beans	—	—	—	—	—	—	2.12 ± 0.16	2.48 ± 0.22	—	—	—	—	—	—	—	2.26 ± 0.18	—	—	—	—	—	—	—	2.65 ± 0.18
Fried vegetables and lean pork	—	—	—	—	—	—	—	2.00 ± 0.12	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Lentils and cured sausage	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chickpea and vegetable soup	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chicken soup	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Pork filet in sauce	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.45 ± 0.20	3.51 ± 0.32
Veal in sauce	—	—	—	—	—	—	—	1.25 ± 0.17	—	—	—	—	—	—	—	1.62 ± 0.06	—	—	—	—	—	—	—	1.95 ± 0.11
Hake in sauce	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.35 ± 0.20
Fish soup	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Chicken breast	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.10 ± 0.01
Grouper in Spanish sauce	—	—	—	—	—	—	1.90 ± 0.11	2.30± 0.20	—	—	—	—	—	—	1.68 ± 0.07	1.89 ± 0.11	—	—	—	—	—	—	2.97 ± 0.25	3.13 ± 0.00
Meatballs	—	—	—	—	—	—	2.10 ± 0.16	2.30± 0.20	—	—	—	—	—	—	2.25 ± 0.20	2.30 ± 0.21	—	—	—	—	—	—	2.14 ± 0.00	2.59 ± 020
Lamb hotpot	—	—	—	—	—	—	2.05 ± 0.13	2.48 ± 0.22	—	—	—	—	—	—	—	1.95 ± 0.12	—	—	—	—	—	—	1.36 ± 0.21	1.78 ± 0.10
“Consommé”	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2.25 ± 0.20	—	—	—	—	—	—	—	—
“Madrid-Cocido”	—	—	—	—	—	—	2.30 ± 0.20	2.85 ± 0.30	—	—	—	—	—	—	—	2.05 ± 0.13	—	—	—	—	—	—	—	2.67 ± 0.22
Meatballs in sauce	—	—	—	—	—	—	1.68 ± 0.07	2.00 ± 0.12	—	—	—	—	—	—	2.06 ± 0.13	2.36 ± 0.22	—	—	—	—	—	—	2.68 ± 0.22	3.58 ± 0.33
Grilled lamb chop	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Data represent mean ± standard derivation of three replications.

[a] Enterobacteriaceae, *St. aureus*, *Streptococcus* D, anaerobic, total coliforms, *E. coli*, *Salmonella-Shigella* and *Listeria* resulted negative.

[b] (-) Not detected.

in any of the different packagings. However, mould, which is an important microbial problem limiting the shelf-life of high moisture products, can tolerate a low oxygen concentration and is simply delayed by MAP (Smith, 1996).

Any decrease in the effectiveness of the packaging used greatly affects the safety associated with the consumption of foods. Major concerns arise from environmentally borne micro-organisms and so sanitary handling conditions must be ensured throughout the processing chain (Pintado and Malcata, 2000).

Initial bacterial population, temperature and gas are important factors which affect shelf-life. It has also been reported that nitrogen, an inert tasteless gas used as filler gas to replace O_2 (Church and Parsons, 1995), and CO_2 are probably related with a reduction in intra and extracellular pH and the direct inhibition of enzymatic processes. This effect generally increases at lower temperature since solubility of the CO_2 is enhanced, delaying spoilage by psychrotrophic, aerobic and Gram-negative bacteria (López-Caballero et al., 2000; Özogul et al., 2000).

Table 4 shows the proximate composition during 29 days storage of the ready to eat foods (lentil soup, meat stew and meat-legume and vegetable soup) packaged in modified atmospheres compared with conventional packaging in air at 'day 0' (the day of manufacture) as control. The data for vacuum packaging showed minimal differences ($P<0.05$) (data not shown) when compared with modified atmosphere. Both industrial packaging methods maintained a good quality in the parameters studied.

Several studies have been published on moisture content and antioxidant components, both of which were shown to be better preserved by MAP during 2 or 3 weeks storage at 2°C (Howard and Hernández-Brenes, 1998; Remón et al., 2000).

Having verified that the storage of several cooked foods in vacuum or MAP offered significant improvement as regards microbial counts over conventional packaging it was still unclear which of the two was the most suitable for the types of food being studied. The modified atmosphere provided a more natural appearance, since the vacuum packaging led to small adherences which decreased the foods attractiveness and hindered separation as mentioned by too Church and Parsons (1995).

Subsequently, we evaluated the shelf-life of a list of typical ready to eat cooked foods stored for a maximum of 29 days in MAP. The counts of the microbial groups are summarized in Table 5. After 21- and 29-day storage period, mean aerobic mesophilic populations varied from 1.05 to 3.58 log cfu/g, psychotrophic micro-organisms varied from 1.62 to 2.78 log cfu/g and yeast/mould from 1.10 to 3.58 log cfu/g, according to the product.

In all the cases studied, the potential risk of spoilage through micro-organism growth appeared minimal and we concluded that such ready to eat foods packed in vacuum or modified atmosphere could safely carry a 'use by' date of 29 days post-manufacture on the packaging (under the responsibility of the processors). The high barrier film in conjunction with strict temperature control and good sanitary handling ensured minimal changes in proximate composition and microbial condition, thus satisfying consumer demand for preservative-free foods with a long shelf-life.

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