MICROBIOLOGICAL AND NUTRITIONAL QUALITY OF SOUS VIDE OR TRADITIONALLY PROCESSED FISH: INFLUENCE OF FAT CONTENT

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ABSTRACT

Sous vide technology, which implies vacuum packaging, pasteurization and storage at a chill temperature, also implies a challenge to obtain a balance between an extended shelf life and microbiological safety of the product with a fresh-like appearance and a high nutritional value. The aim of this work was to determine the effect of fat content of two fish species (salmon and trout) on survival of mesophilic, psychrotrophic, Enterobacteriaceae*,* Micrococaceae *and anaerobes during sous vide cooking and their control during chilled storage for 3, 20 and 45 days. Proximate and fatty acid composition was also determined using a traditional cooking method. Analyses were undertaken comparatively with the same culinary technique for sous vide and traditionally cooked fish. Higher fat levels in all products resulted in fewer microbial reductions. In order to establish adequate pasteurization (temperature/time) combinations for the assurance of microbial quality and shelf life of sous vide processed fish, fat content and fatty acid composition of food should be considered.*

INTRODUCTION

Spain, Norway and Portugal are the highest consumers of fish in Europe, 71 g per person per day (Varela *et al.* 1991), which is much higher than that of the rest of the European countries, in spite of the fact that an increase in their consumption has also been observed in the last few years.

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This change in food habits has been caused by the spreading of the beneficial effects of fish consumption on health because of its high protein quality and content similar to meat and its high content in hydrosoluble and liposoluble vitamins, minerals and polyunsaturated fatty acids of the n-3 family (PUFA n-3). It is now known that these fatty acids can play an important role in the prevention and treatment of coronary heart disease, thanks to their hipolipidemic, antiaggregatory and vasodilatation effects, as well as their potential beneficial influence on arthritis, asthma and other inflammatory or self-immune illnesses or certain types of cancer (Sanchez-Muniz *et al.* 1991; Simopoulus 1998). In fact, Nutrition Recommendations in many countries suggest an increased consumption of fish and seafood. However, fish and fish products are vulnerable to various biochemical, physical and microbial forms of deterioration throughout the production chain (catch to retail sale). Moreover, a substantial part of the quality of fresh or processed fish can often be lost at the consumer level after wholesale or retail sale unless stringent preservative measures are taken. Unfortunately, these measures also have a negative impact on the natural and fresh-like appearance (sensory quality) of fish. In order to increase the average amount of fish eaten at home, good quality seafood that is well prepared and conveniently packed should be available (Schellekens 1996).

The market of refrigerated processed food (including sous vide products) of extended durability has increased over the last 5–10 years. In Spain, 145.000 tons of these products were sold in 1997, with an annual increase of 5%. This trend is a result of an increase in consumer demand for fresh and high-quality food. Moreover, there is a higher degree of convenience as these products do not need thawing, are easier to prepare and their taste and texture is much closer to the fresh product.

Sous vide or vacuum-cooked fish is defined as "raw materials or raw materials with intermediate foods, that are cooked under controlled conditions of temperature and time inside heat-stable vacuum pouches" (Schellekens and Martens 1992). Sous vide foods are typically heated at relatively mild temperatures for a long period of time. After heating, the products are quickly cooled and kept in chilled storage (1–4C). The shelf life of sous vide products ranges from 6 to 42 days.

Fish is a popular ingredient in sous vide cooked food products as it keeps its intrinsic qualities (preservation of seafood's natural and fresh-like appearance and extension of its shelf life). However, in sous vide cooked fish very low temperatures of pasteurization are used, which is why the control of surviving microorganisms is an important safety aspect.

The three main factors that determine the microbiological safety of sous vide products are: (1) intensity of heat treatment and duration; (2) temperature reached by rapidity of cooling; and (3) control of chilled storage (temperature and time).

Ben Embarek and Huss (1993) and García-Palacios (1999) have studied the microbiological quality of sous vide cooked food. However, there is little information available on the nutritional and microbiological aspects of sous vide cooked fish, salmon, cod, mackerel and horse-mackerel being the main species studied. There is a great variability in the industrial equipment used to carry out the pasteurization process and in the temperature (60–80C core) and time used (5–60 min). The refrigeration storage temperature used in almost all studies was 2–3C (Bergslien 1996; García-Palacios 1999).

The main health risk in sous vide fish is type E *Clostridium botulinum* (Meng and Genigeorgis 1994). Most studies have focused on seafood adjuvants in search of an additional experimental hurdle form to thermal treatment to minimize potential risks from fish if not properly refrigerated during the distribution phase (Olson 1990; Tolstoy 1991). Over the last few years, *Listeria monocytogenes*, a psychrotrophic emergent pathogen, has been studied. This pathogen, like other microorganisms, must be taken into account when dealing with health risks in this type of food. Other pathogenic bacteria (*Bacillus cereus* and *Clostridium perfringens*) are regarded as contaminants from ingredients, handling of raw materials or hygienic production conditions.

According to the codes of Hygiene of the Advisory Committee on the Microbiological Safety of Food (UK) for cook-chill products with an extended shelf life of more than 10 days and up to 42 days (≤3C), a heat treatment of 90C for 10 min or equivalent ($P_{90} \geq 10$) and strict chill conditions are required to control *C. botulinum* risks. In order to eliminate nonsporeforming pathogens such as *L. monocytogenes*, which is the most heat resistant, a heat treatment of 70C for 2 min ($P_{70} \geq 2$) is sufficient. An adequate heat treatment must be reached to yield at least a six log cycle reduction in the number of psychrotrophic strains of *C. botulinum* type E spores and *L. monocytogenes*.

Some studies carried out by the authors mentioned have also dealt with the hygienic quality of sous vide fish in total viable counts (TVC) on Iron Agar or Plate Count Agar (PCA). The microbiological acceptance limit for human consumption must be confined to less than five log (cfu)/g products (French legislation). The microbiological changes reported vary from 10^5 to 10^8 cfu/g of TVC and the days of microbial stability or shelf life of fish products reported by different authors vary from 7 to 49 days.

There are few data published on the nutritional aspects of sous vide cooked fish and obviously more accurate data on the acceptability of minimally processed foods by consumers are needed. Only Ghazala *et al.* (1996) have analyzed the proximate composition and stability of fatty acids extracted from seal meat and compared sous vide cooking (65 and 85C) with conventional cooking (100C). In turn, Watier (1988) has studied the stability of vitamins B in sous vide salmon.

The nature of food (fat content, pH, aw, presence of osmoprotectans, essential amino acids and lytic enzymes) is an important determinant of the lethality of a heat treatment and also of the possibility of pathogen growth (Schellekens 1996). It would thus be important to study the impact of each factor on the growth and microbial inactivation to establish additional hurdles when the first barriers (temperature adequate refrigeration) in sous vide food fail.

Gorris (1995) studied the influence of various food components on the growth and survival of pathogens (*L. monocytogenes* and nonproteolytic *C. botulinum*) in ready-to-eat-foods. His findings are important in the assessment of the heat treatments required to ensure the safety of sous vide foods, particularly seafood.

The aim of this work was to study the microbiological quality, proximate and fatty acid composition and possible correlation between fat content and microbial growth in processed fish (salmon and trout) using traditional or sous vide cooking methods and subsequent chilled storage for 3, 20 and 45 days.

MATERIALS AND METHODS

Fifteen kilograms of rainbow trout purchased in different retail stores in León, Spain, were used in this study. The fish were cleaned, filleted, salted and divided into three batches: raw trout (T), trout cooked in its own juice using the traditional method and stored at 4C for 3 days (Tt3) and raw trout which underwent the sous vide process, was vacuum packed using a vacuum sealing machine (TECNOTRIP, Barcelona, Spain), pasteurized in a steam oven (SURDRY A-142, Bilbao, Spain) (90C for 10 min), cooled in a blast cooler (MATACHANA, Barcelona, Spain) and stored at 4C for 3 (Tv3), 20 (Tv20) and 45 days (Tv45).

The treatments were carried out using the industrial equipment of the "de Celis Catering Company" León, Spain, (Fig. 1).

A same study was carried out on fish with a higher fat content: raw salmon slices (S) traditionally cooked (St3) and subjected to the sous vide method and refrigerated at 4C for 3 (Sv3), 20 (Sv20) and 45 (Sv45) days.

The analyses were carried out on three salmon batches and three trout batches and consisted of:

- pH; determined using a pH meter CRISON BASIC 20 (Crison Instruments, Barcelona, Spain) (AOAC 1998).
- a_w; determined using a. Aqua LabTM 2000 (Decagon, Inc., USA) (AOAC 1995).

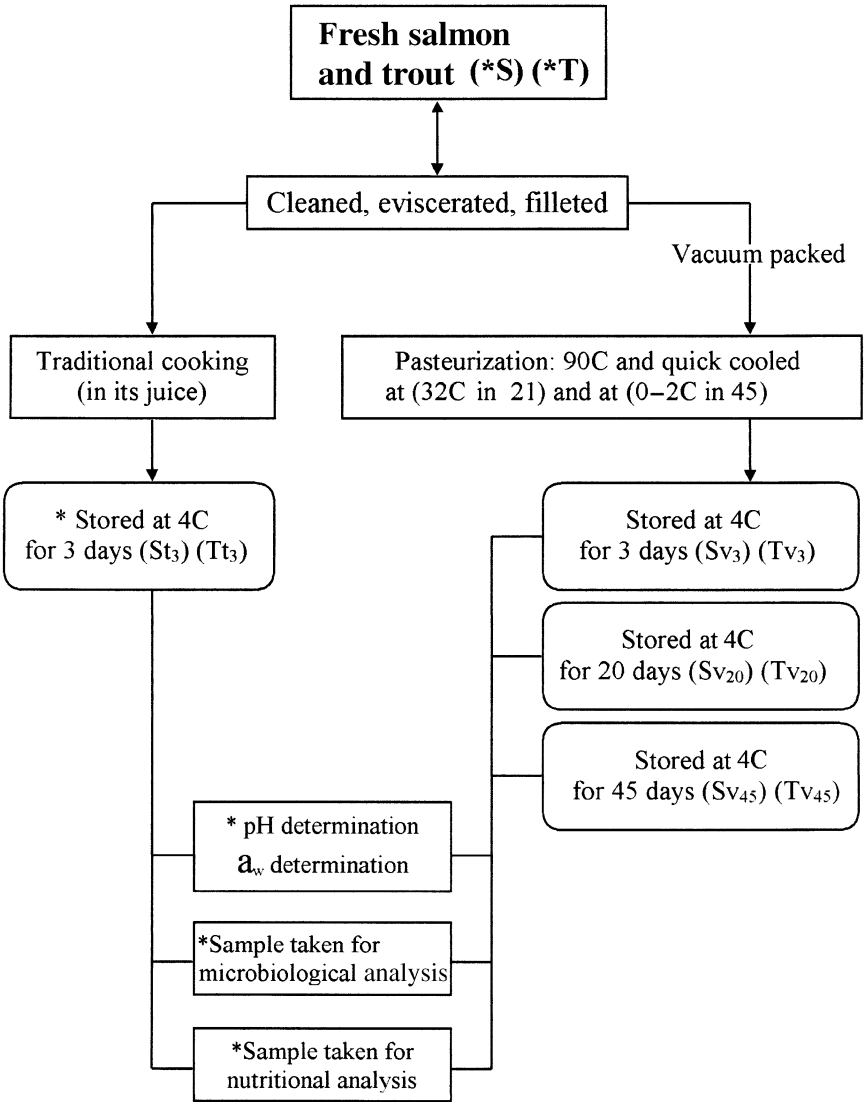

FIG. 1. SAMPLE PREPARATIONS AND PROCESSING

Microbiological Analyses

With the aid of a sterile scalpel, 25 g of sample was removed and placed in a stomacher bag containing 225 mL of 0.1% (w/v) sterile peptone water (Unipath Ltd, Basingstoke, UK). Samples were homogenized using a 400

Stomacher model (A.J. Seward, London) for 2 min. Then, decimal dilutions were carried out using the same diluent.

- Aerobic mesophilic microorganisms were counted on PCA (Unipath Ltd) for duplicate plates incubated for 72 h at 30C (ICMSF 1978).
- Psychrotrophic microorganisms were counted on PCA (Unipath Ltd) incubated at 7C for 10 days (Gilliland *et al.* 1984).
- For *Enterobacteriaceae* enumeration, duplicate 1 mL pour plates of Violet Red Bile Glucose Agar (Unipath Ltd) with overlay were prepared using appropriate dilutions. The plates were incubated at 30C for 24 h and then counted (ICMSF 1978).
- The numeration of *Micrococcaceae* and *Staphylococcus aureus* were carried out using the surface plating procedure on Baird-Parker (Unipath Ltd) medium with incubation at 35C for 48 h (ICMSF 1978).
- Anaerobes counts were carried out by deep seeding in PCA medium (Unipath Ltd) and then incubated in anaerobic jar at 35C for 48 h (ICMSF 1978).
- *Escherichia coli* and *Salmonella* determination was carried out in Violet Red Bile Agar (Unipath, Ltd) (ICMSF 1978) and by the Iso-Grid Hydrophobic Grid membrane Filter (Iso Grid HGMF™ QA Laboratory, Toronto, Ontario) procedure.

Proximate Composition

- *Water content:* five homogeneous samples (3 g) were dried at 100C to constant weight (method 240.03; AOAC 1980).
- *Protein content:* was calculated based on six homogeneous freeze-dried samples from Kjeldahl nitrogen using a 6.25 conversion factor (AOAC 1984).
- *Total fat content:* six homogeneous freeze-dried samples (0.5 g) were extracted with petroleum ether (BP 40–60), in an extracting unit (Soxtec System 1040 Tecator, Sweden). Ether-extracted fat was gravimetrically evaluated.
- *Ash content:* it was determined by heating at 500C to constant weight using a muffle furnace (method 310.12; AOAC 1975).
- *Fatty Acid Composition:* fish fat was extracted following the Bligh and Dyer method (1959) and was saponified with 0.5N of sodium hydroxide in methanol, and then methylated following the Metcalfe *et al.* method (1966). Fatty acid methyl esters of fish fat were analyzed by gas chromatography. A Hewlett-Packard 6890A instrument with mass detector HP5973A was used.

Statistical Analysis

The data were analyzed using one-way analysis of variance (ANOVA). The Newman Keuls test was used to compare means when a significant variation was highlighted by ANOVA. Significance was established at $P < 0.05$ level.

RESULTS AND DISCUSSION

Moisture content in sous vide processed salmon (Sv3) was significantly ($P < 0.05$) lower than in the raw sample (Table 1). However, as percent fat and ash content increased, apparent protein content dropped. The exchange of water and fat in sous vide processing are inversely correlated; this phenomenon has been explained by various authors for other seafood (Mai *et al.* 1978; Beamonte and Castrillon 1989). Nevertheless, it should be taken into account that this processing was carried out without adding oil. Throughout the 45 storage days of sous vide processed salmon, the end of the shelf life, water content increased significantly, lipid content decreased and the protein content of salmon increased. This leads us to think that different exchanges or inter-

TABLE 1.
EFFECT OF TRADITIONAL OR SOUS VIDE COOKING AND SUBSEQUENT STORAGE ON THE PROXIMATE COMPOSITION OF TROUT FILLETS AND SALMON SLICES (G/100 G EDIBLE PORTION)

	Moisture	Protein	Fat	Ash
S	66.60 ± 0.70	18.13 ± 0.37	13.71 ± 0.14	1.12 ± 0.03
St3	63.10 ± 0.81*	19.94 ± 0.16*	15.12 ± 0.31*	1.07 ± 0.10
Sv3	61.44 ± 0.46*†	15.49 ± 0.50*†	19.66 ± 0.42*†	1.27 ± 0.17*†
Sv20	63.58 ± 0.60*‡	20.43 ± 0.22*‡	15.33 ± 0.15*‡	1.04 ± 0.08*
Sv45	62.78 ± 0.57*‡	20.17 ± 0.10*‡	15.88 ± 0.21*‡	1.33 ± 0.07*
T	75.27 ± 0.46§	16.04 ± 0.52§	7.03 ± 0.48§	1.29 ± 0.03§
Tt3	70.85 ± 0.28*§	20.01 ± 0.65*	4.86 ± 0.11*§	1.38 ± 0.09*§
Tv3	69.85 ± 0.09*†§	20.15 ± 0.07*§	7.45 ± 0.22*§	1.53 ± 0.05*§
Tv20	69.56 ± 0.38*§	21.90 ± 0.07*‡§	8.26 ± 0.18*‡§	1.57 ± 0.04*§
Tv45	68.78 ± 0.39*‡§	21.99 ± 0.26*‡§	7.73 ± 0.17*‡§	1.45 ± 0.06*‡§

Values are means ± standard deviations of six homogeneous samples.
T or S: raw trout or salmon; Tr3 or St3, trout or salmon processed by the traditional method after a storage of 3 days; Tv20 or Sv20, Tv45 or Sv45: trout or salmon processed by the sous vide method after a storage of 20 and 45 days, respectively.
* Significantly different ($P < 0.05$) (*) vs the same raw fish species; (†) vs the same fish species cooked using the traditional method; (‡) vs the same fish species stored for 20 days; (§) vs salmon submitted to the same process and the same storage time.

actions among nutrients take place in sous vide processed salmon during long storage periods (20 – Sv20 – or 45 – Sv45 – days).

If fish is cooked in the traditional way (St3), its protein content is higher and it has less fat and ash compared with sous vide cooking (Table 1).

Water and nutrient content of raw, traditionally cooked trout (T) (Tt3) sous vide cooked trout (Tv3) and trout stored for 20 and 45 days (Tv20 and Tv45) are shown in Table 1 and were similar to those reported by others authors (Moreiras *et al.* 1992; Souci *et al.* 1998). After sous vide processing, the flesh had lost 5% of its water (relative value) and this loss increased up to 7% after 45 days of storage. These data are in good agreement with those reported by García-Arias (1989) and Moreiras *et al.* (1992) for other species of fish and other processes.

The decrease in water content resulted in a significant increase ($P < 0.05$) in percent protein content after both cooking methods and upon subsequent storage of sous vide trout for 20 and 45 days.

There was an increase in fat levels after the sous vide treatment (Tv3) and after 20 days in storage (Tv20). Nevertheless, when it was stored for another 25 days, there was a significant decrease in fat content. However, the amount of lipids in trout cooked in the traditional way (Tt3) was significantly lower than in trout cooked using sous vide process.

The ash content increased significantly in trout cooked by both methods. Nevertheless, this increase was lower when trout was cooked using the traditional method and at the last stage of storage (20–45 days).

The fatty acid composition of both fish was significantly different (Table 2). Trout has a higher saturated and total polyunsaturated fatty acids content and a lower monounsaturated total content than salmon. These differences are mainly due to C16 : 0 for saturated fatty acids (SFA), to C22 : 1 for monounsaturated fatty acids (MUFA) and to C18 : 2 and C22 : 6 for PUFA, with values of 13.78 ± 0.09 vs. 8.53 ± 0.05 in salmon, which causes a greater N-3/N-6 ratio in trout, 1.60, compared with that of salmon, 1.26.

The total content of SFA and MUFA did not vary either with the processes studied or throughout the 45 days of storage in either fish (Fig. 2). However, the total PUFA content of trout fell significantly throughout storage. This drop caused a decrease in the P/S ratio and an increase in the n-3/n-6 ratio because of a significant drop in linolenic (C18 : 2) and arachidonic (C20 : 4) acid, both belonging to the family n-6 (data not published). The stability of docoxahexanoic (C22 : 6) acid during sous vide treatment and storage for 45 days in refrigeration must be pointed out in content of total SFA, MUFA, PUFA, P/S or n-3/n-6 ratios, which did not vary for salmon during either the processing or storage period.

As regards sanitary quality, it must be pointed out that in all the samples of both raw fish (S and T) processed by both methods and stored for 20 (Sv20,

TABLE 2.
FAT CONTENT AND FATTY ACID COMPOSITION OF RAW SALMON AND TROUT (G FATTY ACID/100 G TOTAL FATTY ACID)

	Salmon	Trout
Fat content	13.71 ± 0.14	7.03 ± 0.48
Fatty acid composition		
C14 : 0	4.88 ± 0.03	4.44 ± 0.04*
C16 : 0	12.72 ± 0.00	16.38 ± 0.01*
C16 : 1 n-7	6.12 ± 0.03	5.53 ± 0.02*
C18 : 0	2.37 ± 0.02	3.22 ± 0.02*
C18 : 1 n-9	16.29 ± 0.02	19.37 ± 0.10*
C18 : 1	2.91 ± 0.02	0.41 ± 0.04*
C18 : 2 n-6	3.65 ± 0.02	8.58 ± 0.00*
C18 : 3 n-3	1.10 ± 0.00	1.96 ± 0.01*
C20 : 1 n-9	1.86 ± 0.02	1.39 ± 0.02*
C20 : 4 n-6	10.26 ± 0.06	5.13 ± 0.33*
C20 : 5 n-3	5.72 ± 0.02	4.36 ± 0.02*
C22 : 1 n-9	10.75 ± 0.00	5.07 ± 0.03*
C22 : 5 n-3	2.22 ± 0.00	1.81 ± 0.05*
C22 : 6 n-3	8.53 ± 0.05	13.78 ± 0.09*
SFA total	19.97	24.04
MUFA total	37.93	31.77
PUFA total	31.48	35.62
P/S ratio	1.58	1.48
n-3/n-6 ratio	1.26	1.60

* significantly different ($P < 0.05$) vs salmon. SFA: Saturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids.

Tv20) and 45 (Sv45, Tv45) days, *Salmonella* spp. enterotoxigenic *Staphylococcus* and *E. coli* were absent.

The evolution of the microbial contamination in both fish can be seen in Figures 3–7. Raw salmon shows a high contamination by mesophilic bacteria above 4 log cfu/g and trout has a high contamination by mesophilic and psychrotrophic microorganisms (counts above 5 log cfu/g).

As regards the behavior of the surviving flora, in both sous vide processed fish refrigerated for 20 days, it was observed that salmon maintained its original level while trout showed a lower mesophilic, psychrotrophic, *Enterobacteriaceae* and *Micrococaceae* counts compared with the same fish stored for 3 days. However, in the last stage of storage (20–45 days) there was a dramatic change in the behavior of the flora in both types of fish. A significant increase in the counts of mesophilic and psychrotrophic was observed in trout samples, while in salmon only a slight increase in the viable mesophilic flora

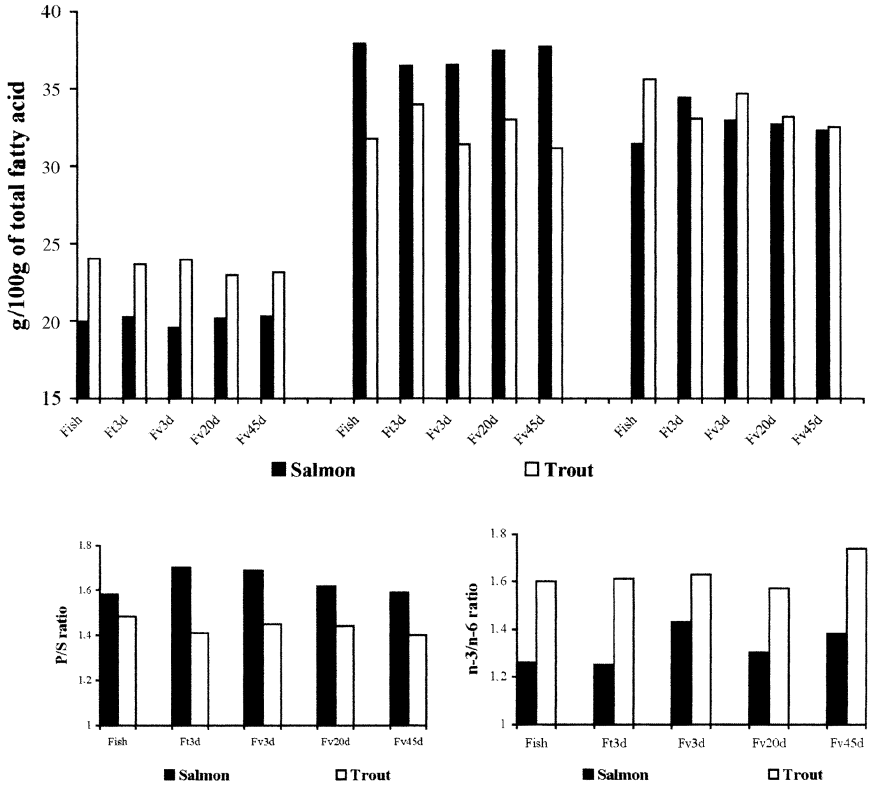

FIG. 2. TOTAL SATURATED FATTY ACIDS, MONOUNSATURATED FATTY ACIDS AND POLYUNSATURATED FATTY ACIDS CONTENTS, P/S AND N-3/N-6 RATIOS OF SALMON AND TROUT PROCESSED USING THE TRADITIONAL AND SOUS VIDE COOKING METHODS

and the *Enterobacteriaceae* was observed. Anaerobic flora was not detected during refrigerated storage in the first 20 days because it was below 10^1 cfu/g (detection limit of the technique used) and only a small increase was observed in the final stage of storage (20–45 days) in salmon samples.

The microbial reduction of mesophilic, psychrotrophic, *Enterobacteriaceae*, *Micrococaceae* and anaerobic counts, and the evolution of pH and a_w in salmon and trout subjected to both cooking methods can be seen in Table 3. No variations in pH and a_w were found, which does not support a different behavior of the microorganism throughout the storage period.

As regards the hygienic quality indices studied in salmon and trout, it can be said that both treatments significantly reduced ($P < 0.05$) microbial

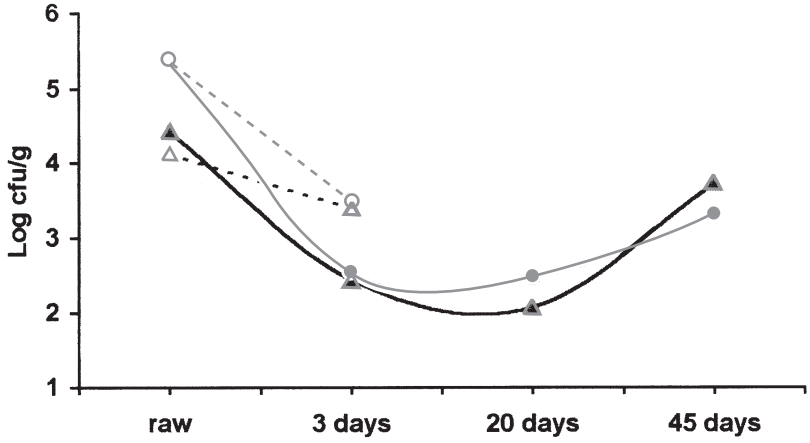

FIG. 3. EVOLUTION OF MESOPHILIC COUNTS IN SALMON AND TROUT PROCESSED BY THE TRADITIONAL AND SOUS VIDE COOKING METHODS
Salmon cooked using the sous vide method (—●—), trout cooked using the sous vide method (—▲—), salmon treated using the traditional method (—○—), trout treated using the traditional method (—△—).

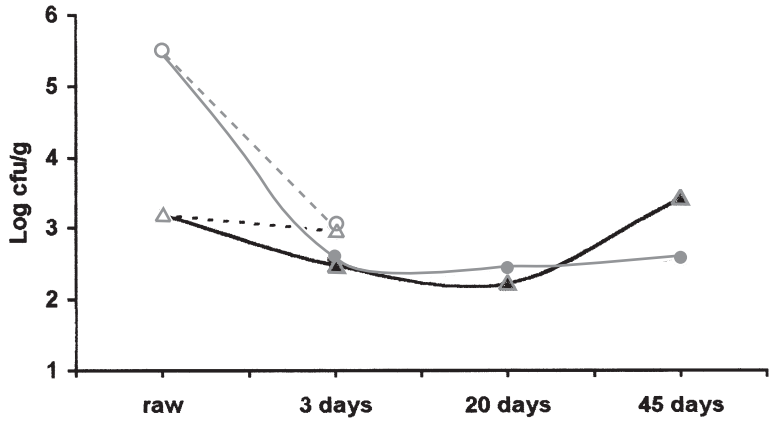

FIG. 4. EVOLUTION OF PSYCHROTROPHIC COUNTS IN SALMON AND TROUT PROCESSED BY THE TRADITIONAL AND SOUS VIDE COOKING METHODS
Salmon cooked using the sous vide method (—●—), trout cooked using the sous vide method (—▲—), salmon treated using the traditional method (—○—), trout treated using the traditional method (—△—).

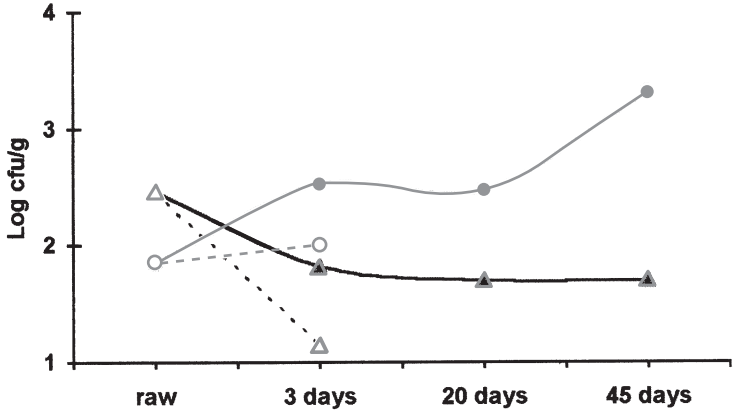

FIG. 5. EVOLUTION OF *ENTEROBACTERIACEAE* COUNTS IN SALMON AND TROUT PROCESSED BY THE TRADITIONAL AND SOUS VIDE COOKING METHODS
Salmon cooked using the sous vide method (–●–), trout cooked using the sous vide method (–▲–), salmon treated using the traditional method (–○–), trout treated using the traditional method (–△–).

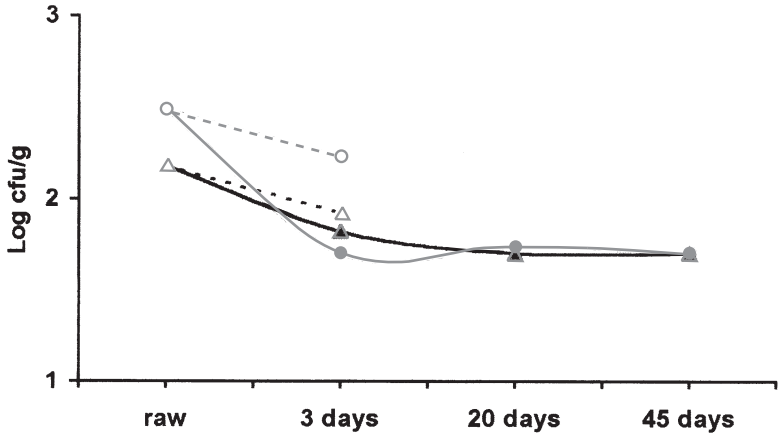

FIG. 6. EVOLUTION OF *MICROCCOCACEAE* COUNTS IN SALMON AND TROUT PROCESSED BY THE TRADITIONAL AND SOUS VIDE COOKING METHODS
Salmon cooked using the sous vide method (–●–), trout cooked using the sous vide method (–▲–), salmon treated using the traditional method (–○–), trout treated using the traditional method (–△–).

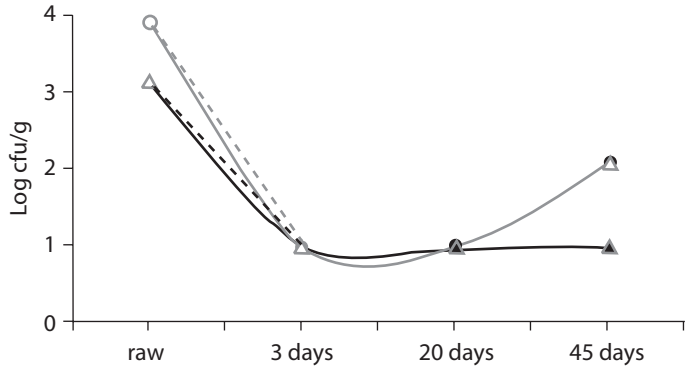

FIG. 7. EVOLUTION OF ANAEROBES COUNTS IN SALMON AND TROUT PROCESSED BY THE TRADITIONAL AND SOUS VIDE COOKING METHODS
Salmon cooked using the sous vide method (—●—), trout cooked using the sous vide method (—▲—), salmon treated using the traditional method (—○—), trout treated using the traditional method (—△—).

contamination, which was to be expected given that these types of processes involve an established heat treatment that destroys nonheat resistant contaminating flora. Microbial reductions in all the indices evaluated, except *Enterobacteriaceae*, were always significantly higher ($P < 0.05$) in sous vide fish (0.5–1 log cfu/g) than in traditional fish and they were significantly higher ($P < 0.05$) in fish with a higher fat content, i.e., salmon (Table 3).

This increase in mesophilic flora throughout storage in sous vide fish with a lower fat content coincide with the results obtained by Rosnes *et al.* (1999) in studies also carried out on two sous vide cooked fish (salmon and cod).

Scientific norms for pasteurization values (temperature/time), cooling rates, storage temperatures and shelf life must be established. One relevant factor to be taken into account and scarcely investigated is the composition of food (Schellekens 1996; Gibbs 1999).

Studies on heat resistance carried out on fish with different fat contents and in broth have concluded that a greater fat content in fish (i.e., 20% as opposed to 0.5%) acts as a protecting agent in the heat resistance of pathogenic microorganisms such as *L. monocytogenes* and *E. coli* (Mackey and Bratchell 1989; Ben Embarek and Huss 1993). Previously, Zuccaro *et al.* (1951) had demostrated that fat can act as a protecting agent and that microorganisms can cross the interface between the fat and aqueous phases to grow out under more favorable conditions.

However (Mackey *et al.* 1990; Buncic *et al.* 1992) two other studies carried out on ground beef with the same percentage of fat (20%) showed that no significant protective effect of fat could be demonstrated.

TABLE 3.
REDUCTIONS OF MICROBIAL GROUPS (LOG_{10} CFU/G), PH a_W AND FAT DATA IN TRADITIONAL AND SOUS VIDE COOKED SALMON OR TROUT

			pH	a_w	Fat	Microbial reductions				
						Mesophilic	Psychrotrophic	*Enterobacteriaceeae*	*Microccocaceae*	Anaerobes
	Raw	S	6.33	0.9953	13.71	–	–	–	–	–
		T	6.56	0.9973	7.03	–	–	–	–	–
Treatment	Traditional	St3	6.51	0.9985	15.12	1.90	2.44	1.01	0.26	>2.89
		Tt3	6.61	0.9958	4.86	0.73†	0.23†	1.32	0.26	>2.12
	Sous vide	Sv3	6.47	1.0000	19.66	2.87*	2.91*	0.97	0.79*	>2.89
		Tv3	6.56	0.9923	7.45	1.68*†	0.71*†	1.58*	0.36*†	>2.12
Storage	3–20 days	Sv20	6.43	0.9957	15.33	0.06‡	0.14‡	0.11‡	–0.04‡	ND
		Tv20	6.61	1.0000	8.26	0.37	0.18	0.18	0.12	ND
	20–45 days	Sv45	6.52	0.9957	15.88	–0.84	–0.12	–0.20	0.04	<–2.11
		Tv45	6.51	0.9980	7.73	–1.65§	–1.20§	–0.73§	0.00§	ND

Indicate significantly differences ($P < 0.05$); (*) with respect to the same fish species cooked using the traditional method; (†) with respect to St3 or Sv3, respectively; (‡) with respect to Tv20; (§) with respect to Sv45.

The reason proposed by these authors is that fat was added to ground meat and this may not have the same protective effect as fatty meat tissue. However, it must also be considered that fish and meat are different.

CONCLUSIONS

Trout cooked by the sous vide method and stored for 20 and 45 days kept the lipid content of the raw species. However, when cooked by the traditional method, the fat content fell dramatically. Traditional and sous vide cooking maintains and even increases the protein and lipid content as well as the fatty acid characteristics of salmon (ω-3).

The proximate composition of fish and particularly its total fat content and fatty acid composition have a significant ($P < 0.05$) influence on microbial survival and development of indicators of hygienic quality during the refrigeration storage (mesophilic, psychrotrophic and *Enterobacteriaceae*) considered according to the microbiological norms for sous vide products established by the French Health Law.

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