

Microbiological Quality of Filled Pasta in Relation to the Nature of Heat Treatment

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ABSTRACT

The microbial population present in 49 samples of Italian industrially processed filled pasta was characterized and its changes during refrigerated storage were evaluated. The most frequently isolated species belonged to the genus *Bacillus*. No pathogenic organisms were isolated from the processed industrial pasta. As a consequence of the diversity of composition and thermal treatment a wide variability was observed (from less than 3 days to more than 1 month) in the shelf life at 4°C of the industrial "fresh filled pasta." However, the results obtained suggested that the shelf life of the processed products depended not only on the number of surviving cells but also on the textural or microstructural changes induced by the heat treatment. Challenge tests using *Staphylococcus aureus* showed that even pasteurization values (P_{70}^{10} , expressed as an equivalent process time, in minutes, necessary to obtain at 70°C the same lethal effect as during the actual process) not exceeding 2 were able to remarkably reduce the cell load of this organism. Subsequent growth of the surviving *S. aureus* cells occurred only at temperatures >7°C, particularly when the water activity (a_w) values were higher than 0.97.

In contrast to the traditional perishable products whose shelf life does not exceed 3 days, packaged "fresh filled pasta" has become a convenience food having a shelf life ranging from 1 to 12 weeks when marketed under refrigeration (11, 12). The definition of "fresh pasta" is, however, equivocal since the attribute "fresh" is used to indicate both nonthermally processed pasta and pasta subjected to various thermal treatments. In fact, according to Italian law, the only criterion is a water content higher than 30% (wt/wt). Spoilage organisms such as thermotolerant fungi, particularly *Rhizopus nigricans* and *Cladosporium cladosporoides*, and spore-forming bacteria belonging to the genus *Bacillus* have been isolated in such thermally processed food (3, 6, 8). On the other hand, hazardous microorganisms, such as *Escherichia coli*, *Staphylococcus aureus*, and more rarely *Listeria monocytogenes* are also associated with handmade fresh pasta (2, 9, 10, 13, 18, 19).

In addition to these products, three principal categories of industrial filled pasta on the Italian market can be identified on the basis of the severity of the thermal treatment. The flow diagram shown in Figure 1 reports the principal steps in the production of different types of filled pasta: thermally untreated ravioli (UN), flash-pasteurized ravioli (B), ravioli subjected to flash pasteurization followed by a second pasteurization after packaging (C), and filled pasta subjected to flash pasteurization and stronger microwave or thermal treatment after packaging (D). The pasteurization values, expressed as an equivalent process time, in minutes, necessary to obtain at 70°C the same lethal effect as during the actual process, calculated for a reference tempera-

ture of 70°C (P_{70}^{10}), can range between 0.5 and 200 min, depending on the processing company and the product (5, 10). Despite general safety improvement, the formulation of the products and the thermal treatments applied are often empirically established. Greater knowledge of the effects of the various thermal treatments on spoilage and pathogenic species survival as well as their subsequent growth can provide a more reliable assessment of the safety and shelf life of fresh filled pasta products.

In this study, the microbial populations surviving various thermal treatments in commercial pasta products belonging to the UN, B, C, and D categories were analyzed and their changes during refrigerated or temperature-abuse storage was evaluated. In addition, challenge tests using *S. aureus* or *E. coli* inoculated before thermal treatments were performed.

MATERIALS AND METHODS

Survey of commercial items. The products analyzed consisted of commercially filled pasta packs differing in the type of filling and thermal treatment. In particular, two types of filled ravioli were considered: ravioli filled with ricotta and vegetables, and ravioli filled with cheese and meat.

Four types of products subjected to different thermal treatments were analyzed: 15 samples of thermally untreated handmade ravioli (UN), 10 samples of flash pasteurized ravioli (B), 10 samples of ravioli subjected to flash pasteurization followed by a second pasteurization after packaging (C), and 14 samples of ravioli subjected to flash pasteurization followed by a stronger microwave treatment after packaging (D). The pasteurization values for the products B, C, and D ranged between 0.5 and 8.67, 10.5 and 64.5, 100 and 200, respectively. Of the 49 samples, 24 were filled with ricotta and spinach (rs) and with 25 meat and cheese (mc). The main ingredients used for the meat and cheese

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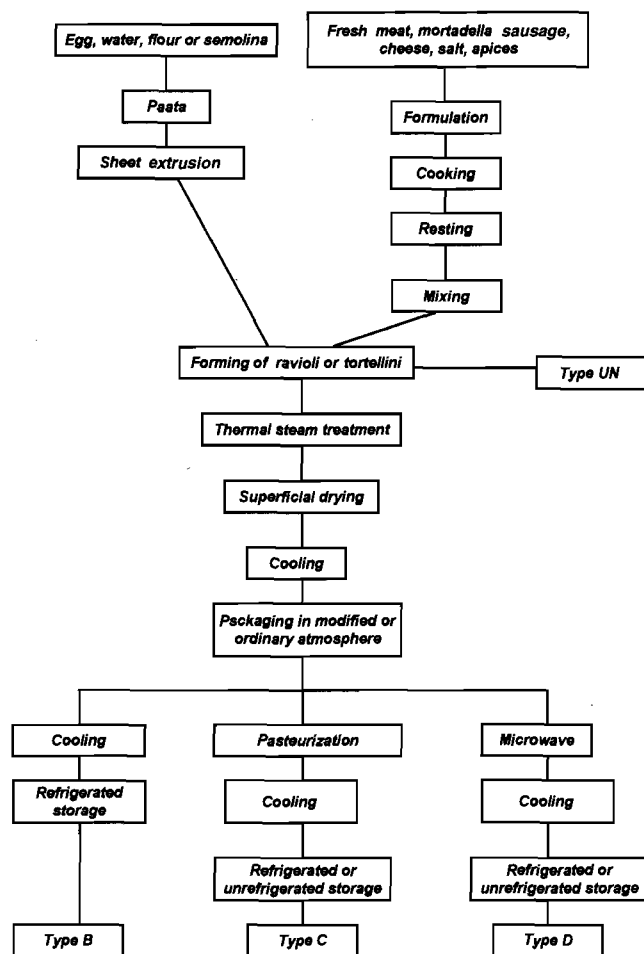


FIGURE 1. Flow chart relative to the production of four types of filled pasta subjected to different heat treatments.

fillings were pork, mortadella sausage (Bologna), ham, Grana cheese, NaCl, glutamate, pepper, and spices. The main ingredients for the ricotta and vegetable filling were ricotta, spinach, eggs, NaCl, and various herbs and spices. The base ingredients of the pasta layer were semolina, fresh eggs (1 egg per 100 g of flour) and water. The relative percentage of the various ingredients and the preparation procedures differed considerably from factory to factory and resulted in products having different water activities.

The pasteurization processes, pasta preparation, and packaging were performed with different equipment and materials at the different factories. The comparison of thermal processes was based on the pasteurization values. The heating profiles of samples were obtained by placing a thermocouple (TESTO thermometer, Testo GmbH & Co, Lanzkirch, Germany) in the geometric center of the sample. The pasteurization values (P_{70}^{10}), expressed as an equivalent process time necessary to obtain, at 70°C, the same lethal effect as during the actual thermal process, were calculated in the product's cold point according to the following equation (7):

$$P_{70}^{10} = \int 10 \exp^{(T-70)/z} dt,$$

where for z a value of 10°C (relative to *Clostridium botulinum*) was used. T was the core temperature of the product in degrees celsius and t was the time in minutes.

All the products, except D types, were packaged in polyethylene (PE) containers under a modified atmosphere whose composition was 50% CO₂ and 50% N₂ or 30% CO₂

and 60% N₂. The D types were packaged under vacuum and then subjected to microwave treatment in microwave ovens for 20 min at 80°C. The microwave ovens and the packaging equipment and material differed according to the companies. All the samples were stored at 4°C and periodically analyzed.

Analysis of the microbial population in the products. A total of 49 different samples was considered. Three replicates of each sample were examined immediately after preparation and thermal treatment and daily during refrigerated storage at ≤4°C. Standard microbiological procedures were used to analyze 10 g of the various samples. After homogenization for 2 min in a Stomacher Lab Blender Model 400 (Seward Medical, England) and appropriate dilutions in peptone water (0.1%), 0.1 ml of the sample was spread on the surface of plate count agar (PCA; Oxoid) plates in duplicate and incubated at 30°C for 48 h to enumerate the total aerobic bacteria. For anaerobic thermoresistant bacteria reinforced clostridial medium plates (RCM; Oxoid) were incubated in anaerobic jars at 37°C for 10 days. Total and fecal coliforms were enumerated on violet red bile agar (Oxoid) incubated for 24 h at 37 and 44°C, respectively. Sabouraud dextrose agar (Oxoid) with chloramphenicol added (100 ppm) incubated at 30°C for 48 h was used for yeasts and molds. The detection of *Salmonella* spp., *L. monocytogenes*, and *S. aureus* was performed according to the methods reported by Andrews (1).

A different number of samples for each type of pasta (UN, B, C, and D) was analyzed for the initial physicochemical properties and for microbiological features. The overall means and the standard deviations obtained by statistical analysis are reported in Table 1.

Evaluation of microbial growth and shelf life in relation to the thermal treatment. In a second experiment, samples of industrial ravioli filled with ricotta and vegetables (indicated as rs) and cheese and meat (indicated as cm) were subjected to different thermal treatments. The surviving aerobic bacteria and their subsequent growth over time and the product shelf life were analyzed. They were either packaged without thermal treatment (indicated as UNrs and UNcm respectively) or subjected to the following treatments: flash pasteurization before packaging (indicated as Brs and Bcm respectively); flash pasteurization followed by a second pasteurization after packaging (respectively indicated as Crs and Ccm). The pasteurization values (P_{70}^{10}) for the products Brs and Bcm were 0.5 and 8.67 respectively, whereas for Crs and Ccm they were 45 and 40. The products were then analyzed daily over time during refrigerated storage at 4°C. A different total number of samples for each type was analyzed because of the different shelf-life extension.

For this experiment the equipment for the automatic double-sheet ravioli production was a Dominioni D 500 (Punto & Pasta Dominioni s.r.l., Lurate Caccivio, Como, Italy). A plant Alimac (Alimac s.r.l., Cittadella, Padova, Italy) equipped with pasteurizer (model TPV-SIT 60) and a cooler (model RAT-SIT 55-5) was used. The pasta was packaged under ordinary atmosphere with an S 100-Tecnovac (Bergamo, Italy).

Characterization of the bacterial isolates. Two or three colonies of each different bacterial morphological type were selected from the PCA or RCM plates of each product. After purification on PCA or RCM, the isolates were grown on PCA slants or in RCM tubes and kept at 4°C. The strains were characterized using the methods of Norris et al. (14) and the Analytical Profile Index (API) 20E, 50CHB and 20A identification strips (BioMerieux, Marcy-l'Etoile, France).

For yeast identification, three colonies with different morphologies were selected from the primary cultures and kept on Sabouraud dextrose agar (Difco) at 4°C until they were identified. The isolates were characterized according to van der Walt and Yarrow (20) and by using the API ATB ID32C system (BioMerieux). Identification was carried out by comparing the test results with the tables of Barnett et al. (4).

Challenge testing. Pure cultures of *S. aureus* DPV1 and *E. coli* DPV 1010 (belonging to the collection of the Dipartimento di Protezione e Valorizzazione Agroalimentare, University of Bologna) were inoculated inside the filled pasta before the thermal treatment.

To prepare the inoculum, *S. aureus* and *E. coli* were grown for 72 h in brain heart infusion at 37°C; individual ravioli were inoculated in aseptic conditions using a sterile syringe before being packaged with an S100 Tecnovac (Bergamo, Italy) in PE and analyzed before and after thermal treatments performed under industrial conditions. The thermal treatments of the products were the following: 2 P_{70}^{10} for the products having an a_w of 0.968, 0.975, or 0.984, inoculated with 4 log CFU of *S. aureus* per g; 200 P_{70}^{10} , in a microwave oven (Milestone Microwave Laboratory System MLS 1,220 MW, FKV, Milan, Italy), for the products having an a_w of 0.984 inoculated with 6.3 log CFU of *S. aureus* per g and 6.1 to 6.5 log CFU of *E. coli* cells per g. The samples were analyzed over time during refrigerated storage at 7°C. In fact, *S. aureus* was unable to grow at temperatures lower than 6 to 7°C.

Baird-Parker medium (Oxoid) with the addition of egg yolk tellurite emulsion (50 ml/liter) incubated at 37°C for 48 h was used for the enumeration of *S. aureus*. Violet red bile agar methylumbelliferyl- β -glucuronide (VRBA-MUG, Biolife) incubated at 42°C was used for *E. coli*.

Curve fitting. The experimental data, expressed as log CFU/g, were modeled using the Gompertz equation modified by Zwietering et al. (22):

$$y = K + Ae^{-e^{-(\mu_{\max}e^A)(\lambda - t) + 1}}$$

where y is expressed as log CFU/g; K is the asymptotic level of bacteria (as log CFU/g) as time decreases indefinitely (i.e., the initial level of bacteria); A is the asymptotic level of bacteria (as log CFU/g) that occurs as time increases indefinitely (final log CFU/g increase); μ_{\max} is the maximum specific growth rate as Δ log CFU $g^{-1}h^{-1}$; λ is the lag time in hours; and t is the storage time in hours.

By means of the Gompertz equation obtained from the various products, the time necessary to reach an arbitrary reference threshold of 7 log CFU/g was predicted. This time was adopted as the shelf-life extension.

a_w and pH determinations. The water activity (a_w) was analyzed with an AquaLab CX2 instrument (Decagon Devices Inc., Pullman, Wash., USA) calibrated with a saturated solution of NaCl and distilled water at a constant temperature. The pH measurements were obtained with a Crison pH meter model 2001 (Crison Instruments, Barcelona) calibrated with two standard solutions buffered at pH 4.00 and 7.02.

RESULTS AND DISCUSSION

Characterization of the initial microbial population and of the physicochemical properties of pasta products. Forty-nine samples of filled pasta produced by different

companies, including handmade products, were analyzed immediately after production. The ingredients of the fillings were meat, ham, cheese, and spinach.

Due to the complexity of the ingredients and their different origins, the microbial species colonizing the UN handmade products were relatively heterogeneous. The gram-negative strains identified belonged to the species *Serratia marcescens*, *Enterobacter sakazakii*, *Pseudomonas paucimobilis*, *Klebsiella pneumoniae*, *E. coli*, and *Pseudomonas* spp. In any case the frequency, as number of isolates of the individual species of gram-negative bacteria per number of total isolates \times 100, was consistently lower than 5%. *L. monocytogenes* was isolated in only one of the commercial samples examined. Yeasts belonging to the species *Candida pelliculosa*, *Candida krusei*, *Rhodotorula* spp., *Candida famata*, *Pichia membranaefaciens* and *Candida* spp. were isolated. The spore-forming aerobic population was more uniform. *Bacillus pumilus* presented a frequency of 38% while the other species, *Bacillus stearothermophilus* (14.3%), *Bacillus coagulans* (14.3%), *Bacillus brevis* (19%), and *Bacillus polymyxa* (9.5%) showed a lower incidence. *Bacillus cereus* was not isolated.

On the other hand, the microbial population surviving the thermal treatments in the products B, C, and D, as well as the strains isolated in the first phases of storage, belonged mainly to the spore-forming genus *Bacillus*. No potentially dangerous species were isolated from the pasteurized pasta, independent of the severity of treatment. *Salmonella*, *L. monocytogenes*, *E. coli*, and *S. aureus* were absent in all the samples examined. Sporadic colonies of *Serratia*, *Erwinia*, and *Pseudomonas* species were isolated. The rare colonies of anaerobic bacteria were the species *Clostridium bif fermentans*, *Clostridium beijerinckii*, and *Clostridium butyricum*. The frequency of the *Bacillus* species in relation to the severity of the treatment in product categories UN, B, and C, is reported in Figure 2. The most frequent species was *B. subtilis*, whose incidence increased with the severity of thermal treatment. *B. alvei* and *B. firmus* were present only in type C pasta products. The occurrence of the various *Bacillus* species does not seem to be specifically associated with the animal or vegetable origin of the filling. All the industrial products, except D types, were packaged under modified atmospheres of different composition. However, no significant differences related to the composition of the atmosphere were observed.

The mean initial values of a_w and pH of the various commercial products in the categories UN, B, C, and D did not present remarkable differences, even though notable differences for individual samples, especially for the a_w , from the mean value were observed (Table 1). However the mean pH of the unprocessed ravioli was lower, probably due to earlier spoilage of the filling. In the processed pasta, the mean initial microbial population was, as expected, dependent on the severity of the thermal process. As previously reported, the composition of the microbial population was modified by the treatment. In fact, no coliforms could be detected in 10 g of products of types B, C, and D after

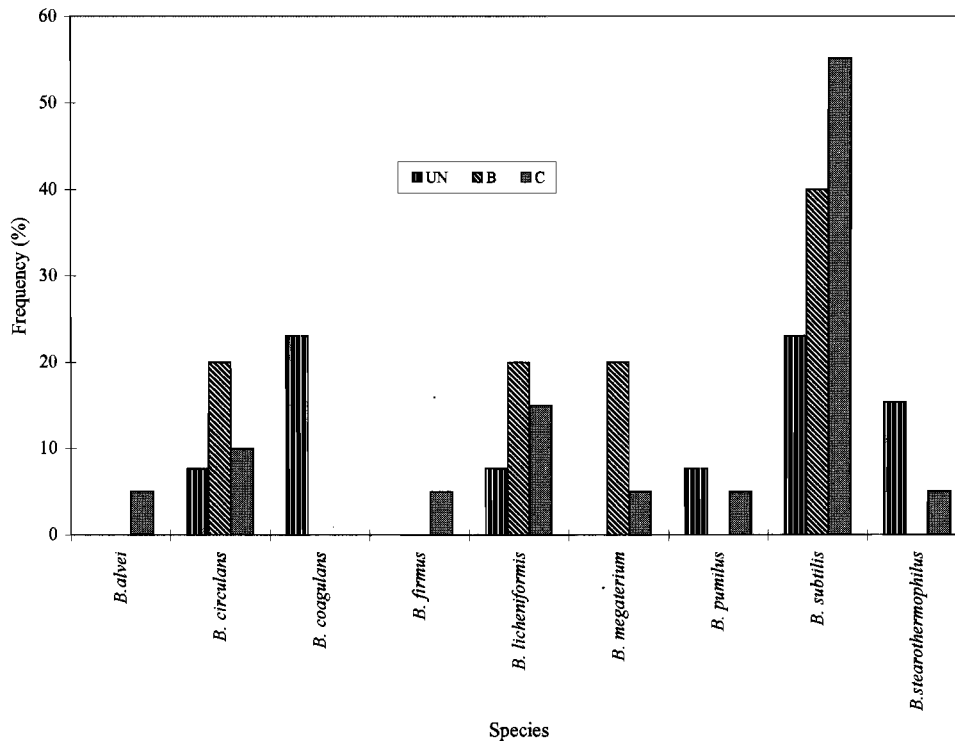


FIGURE 2. Frequency of *Bacillus* species isolated from handmade pasta (UN), pasta subjected to flash pasteurization before packaging (B) and filled pasta subjected to flash pasteurization and to a second pasteurization after packaging (c).

pasteurization. The initial total mesophilic bacteria did not exceed 2.8 log CFU/g in the D type products.

Evaluation of microbial growth and shelf life in relation to the thermal treatments. In order to evaluate the growth dynamics of the surviving population during refrigerated storage, in a second experiment samples of industrial ravioli filled with ricotta and vegetable (indicated as rs) and cheese and meat (indicated as cm) were subjected to different thermal treatments. The surviving aerobic bacteria and their subsequent growth over time and the product shelf life were analyzed. These products were either packaged without thermal treatment (respectively indicated as UNrs and

UNcm) or were subjected to the following treatments: flash pasteurization before packaging (indicated as Brs and Bcm respectively), and flash pasteurization followed by a second pasteurization after packaging (indicated as Crs and Ccm respectively). The pasteurization values (P_{70}^{10}) for the products Brs and Bcm were 0.5 and 8.67 respectively while for Crs and Ccm they were 45 and 40. The products were then analyzed daily over time during refrigerated storage at 4°C.

The growth data, as log CFU/g, of the total mesophilic aerobic bacteria (mainly *Bacillus* species) were analyzed with the modified Gompertz equation. Table 2 reports the

TABLE 1. Mean physicochemical characteristics and mean contamination levels of commercial filled pasta in relation to two types of filling and thermal treatments: (UN) handmade products, (B) filled pasta subjected to flash pasteurization before packaging, (C) filled pasta subjected to flash pasteurization and a second pasteurization after packaging, and (D) filled pasta subjected to flash pasteurization and microwave treatment

Type of pasta ^a	P_{70}^{10b}	pH	a_w		Initial bacterial load (log CFU/g)		
			Filling	Pasta	Total mesophils	Total coliforms	Fecal coliforms
UNrs	0	5.5 ± 0.3	0.946 ± 0.01	0.948 ± 0.007	6.4 ± 1	5.6 ± 0.5	4.6 ± 0.8
UNmc	0	6.1 ± 0.3	0.948 ± 0.006	0.947 ± 0.008	5.4 ± 1.2	4.9 ± 0.6	3.6 ± 0.6
Brs	4.6 ± 4	6.2 ± 0.2	0.965 ± 0.009	0.960 ± 0.008	4.4 ± 0.5	ND ^c	ND
Bmc	4.6 ± 4	6.3 ± 0.09	0.934 ± 0.009	0.935 ± 0.008	2.2 ± 0.8	ND	ND
Crs	37.5 ± 27	6.3 ± 0.09	0.965 ± 0.008	0.966 ± 0.009	2.0 ± 0.3	ND	ND
Cmc	37.5 ± 27	6.2 ± 0.08	0.965 ± 0.008	0.955 ± 0.007	1.9 ± 0.5	ND	ND
Drs	150 ± 50	6.2 ± 0.1	0.936 ± 0.008	0.943 ± 0.009	2.0 ± 0.4	ND	ND
Dmc	150 ± 50	6.2 ± 0.1	0.937 ± 0.009	0.957 ± 0.006	2.0 ± 0.8	ND	ND

^a rs, ricotta and spinach; mc, meat and cheese.

^b P_{70}^{10} , pasteurization value calculated according to the equation $P_{70}^{10} = \int 10 \exp^{(T-70)/z} dt$.

^c ND, not detectable in 10 g.

TABLE 2. Gompertz parameters and shelf life prediction of filled pasta in relation to two types of filling and thermal treatments: (UN) handmade products, (B) filled pasta subjected to flash pasteurization before packaging, (C) filled pasta subjected to flash pasteurization and a second pasteurization after packaging

Type of pasta ^a	P_{70}^{10b}	Bacterial load (CFU/g)		Predicted shelf life (h) ^c
		Initial: K	Maximum: A	
UNrs	0	6.5	7.2	48.0
UNmc	0	4.6	7.1	55.2
Brs	0.5	5.8	7.6	60.0
	8.67	3.5	7.1	91.1
Bmc	4.38	1.8	6.5	410.0
	8.63	1.5	6.7	432.0
Crs	45	1.5	3.3	NA ^d
Cmc	40	1.5	2.6	NA

^a rs, ricotta and spinach; mc, meat and cheese.

^b P_{70}^{10} , pasteurization value calculated according to the equation $P_{70}^{10} = \int 10 \exp^{-(T-70)/z} dt$.

^c Shelf life predicted by solving the Gompertz equation obtained for the various pasta types for any value of 7 log CFU/g and calculating the time necessary to reach this threshold.

^d NA, values did not exceed 3.3 and 2.69 log CFU/g respectively for Crs and Cmc within 30 days.

calculated Gompertz parameters: the initial contamination level (K), the maximum growth extent (A) and the predicted shelf life, expressed as the time (in hours) necessary to attain a threshold level of 7 log CFU/g and calculated by means of the various Gompertz equations obtained.

In the products UN and B, the maximum final cell counts (parameter A) were similar, although the growth rate and, consequently, the shelf-life extension were remarkably different. In the C products, which presented a mean initial cell level of 1.5 log CFU/g, the maximum final growth extent did not exceed 3.3 log CFU/g. Analogously, in the D products the population surviving the thermal treatment (1.5 to 2.8 log CFU/g) tended to decrease during storage at 4°C (data not shown). The low growth extent observed in the D products is not sufficiently accounted for only when viewed in terms of the effectiveness of thermal process. In fact, such a reduced proliferation of the surviving cells in systems having relatively high pH and a_w values suggests that the thermal treatments had induced microstructural modifications of the system which limited cell proliferation.

Protein gelation, as a dependent factor of the ingredients and their proportions, can generate a microstructure with pores on a much smaller scale than bacterial dimensions that can prevent movements of cells and partially limit nutrient diffusion (21).

Challenge tests on B and D types of ravioli. Previous investigations identified *S. aureus* as the most frequently occurring hazardous species in pasta products (16, 17). A challenge test of type B ravioli was set up to investigate the ability of *S. aureus* to survive thermal treatment and grow in commercial meat-filled pasta having three different a_w values. Meat ravioli (type B) previously inoculated with

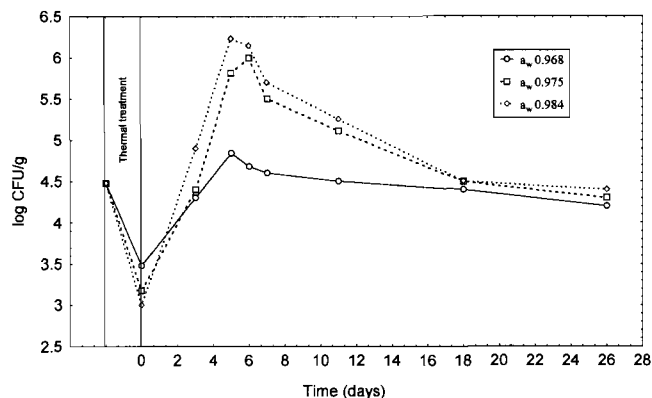


FIGURE 3. Population changes of *Staphylococcus aureus* during storage at 7°C of meat-based ravioli having different a_w values.

about 10^4 CFU of *S. aureus* per g was subjected to a flash thermal treatment equivalent to $2 P_{70}^{10}$ and stored at 7°C.

The flash pasteurization induced a weak reduction in viability as indicated in Figure 3, which also reports the subsequent changes in the population of *S. aureus* at 7°C. The a_w of the system displayed a remarkable effect on the extent of the maximum growth of *S. aureus* which attained a level of 3×10^5 , 1.7×10^6 , and 4.8×10^4 CFU/g after about 4 days in samples having a_w values of 0.975, 0.984, and 0.968 respectively. Refrigeration at temperatures below 6°C prevented the growth of this organism. These results indicate that commercial products processed with the same P_{70}^{10} can present different safety problems depending on the formulation of the filling.

For type D ravioli, the surviving cell level of *S. aureus* in meat-based ravioli individually inoculated with about 6.3 log CFU/g, packaged, and pasteurized in a microwave oven with $200 P_{70}^{10}$ did not exceed 1.5 log CFU/g, independent of the a_w values (data not shown). Moreover, such cells were not able to further proliferate at 7°C and, after a week of refrigerated storage, no viable cells were found in 10 g of product. The ability of the cells to repair the damage of the thermal treatment depended on the storage temperature. In fact, although the cell viability dramatically decreased during storage at 7°C, *S. aureus* was able to grow to a level of 6 log CFU/g when the product was subjected to thermal abuse at 15°C during the first 10 days after processing (data not shown). According to Notermans and Mead (15), a cell level between 5 and 6 log CFU of *S. aureus* per g is sufficient for the production of a quantity of toxin causing disease symptoms. On the other hand, when the packs were temperature abused after 10 to 15 days following processing, the cells could no longer repair the heat treatment damage or grow.

In order to compare the influence of the filling formulation on the thermotolerance of *S. aureus* and *E. coli*, four types of pasta filled with mushroom and cheese, ricotta and spinach, mixed vegetables, or cheese and meat were inoculated with 6.2 to 6.4 or 6.1 to 6.5 log CFU respectively of *E. coli* and *S. aureus* per g and subjected to $200 P_{70}^{10}$ in a microwave oven. The mean core temperature of the ravioli was 80°C. The lowest decimal reduction times for *E. coli* and *S. aureus* at 80°C were observed when they were

TABLE 3. Effect of filling composition on the D_{80} of *Staphylococcus aureus* and *Escherichia coli*

Ravioli filling	D_{80}	
	<i>E. coli</i>	<i>S. aureus</i>
Mushrooms and cheese	3.24	8.33
Spinach and ricotta	3.08	6.66
Meat and cheese	2.05	3.77
Pesto	3.08	6.47

inoculated in meat and cheese-filled ravioli (Table 3), thus confirming that the composition of the latter filling was better at preventing the growth, survival, and subsequent proliferation.

The results of this survey of some microbiological and physicochemical features of a large number of filled pasta items produced by different companies showed great variability, due to the effects of the different treatments applied. As a consequence of a different decontamination level and of the changes of the microenvironmental conditions, the shelf life of the heat-processed pasta (B, C, and D) ranged from 5 to more than 90 days, although the products on the basis of water content could all be included in the "fresh pasta" category. With respect to the handmade filled pasta, the industrial pasta was characterized by a relatively uniform microbial population. The more frequent species were *B. alvei*, *B. circulans*, *B. firmus*, *B. licheniformis*, *B. megaterium*, *B. pumilus*, *B. subtilis*, and *B. stearothermophilus*. No pathogenic or toxinogenic species were found, except for *L. monocytogenes* isolated from one sample. However, challenge testing with *S. aureus* showed that it was able to survive the thermal treatments but it attained a level exceeding 10^5 CFU/g only in the flash pasteurized samples after 5 to 6 days at $7 \pm 2^\circ\text{C}$, when the shelf life of the products could be considered to have already expired (Table 2).

An hypothesis suggested by this work is that the shelf-life extension of the processed products depends not only on the surviving cell number, as a consequence of the P_{70}^{10} adopted, and on the a_w value, but also on the textural or micro-structural changes and in particular on protein gelation induced by the thermal treatment. The significance of the changes induced by such flash or mild thermal processes to the properties and water relationships of the naturally occurring or added proteins as well as to the filling microstructure should be more thoroughly investigated.

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