SHELF-LIFE OF FRESH FILLED PASTA. HAZARD ANALYSIS AND CRITICAL CONTROL POINTS OF THE MANUFACTURING PROCESS AND HOUSEHOLD PRACTICES

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ABSTRACT

Hazard Analysis and Critical Control Point (HACCP) approach was used to assess the safety of a fresh filling pasta (ricotta-filled ravioli) manufacturing plant in La Plata region, Argentina. Household practices like cooking and holding meals before serving were also evaluated. Samples of ricotta, main raw material of the filling, showed colony counts of Enterobacteriaceae *total microorganisms, molds and yeasts of* (5×10^3-1×10^5), ($1x10^5$ -$1x10^8$) *and* 1×10^5 *CFU/g, respectively.* E. coli *was also detected in one out of five samples, suggesting a hazardous condition of this raw material.* Salmonella spp. *was not isolated from any of the dough samples tested. Ricotta filling showed high colony counts of mesophilic microorganisms* (6×10^6 *CFU/g),* Enterobacteriaceae (1×10^5 - 1×10^6 *CFU/g), while* E. Coli *was detected in 20% of the samples. Counts of* B. cereus, S. aureus *were less than* 1×10^2 *CFU/g in the analyzed materials. In the finished product (ricotta-filled ravioli), the colony counts of mesophilic microorganisms and* Enterobacteriaceae *were* 3×10^8 *and* 8×10^5 *CFU/g, respectively. Critical Control Points found were cooking and holding time before serving. The implementation of suggested corrections allowed the microbial quality of the final product (ricotta-filled ravioli) to be improved considerably. The growth of total microbial counts during refrigerated storage (0, 4, 8 and 10C) were measured and the shelf-life of ricotta and ricotta-filled ravioli was calculated.*

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INTRODUCTION

The purpose of the HACCP (Hazard Analysis and Critical Control Points) in food production is to analyze the potential hazards associated with each processing step to evaluate their risk and, thereby, to identify those operations in which corrective actions will be required. Experience has demonstrated that this approach improves food safety (Bryan *et al.* 1992). Hazards vary from one product to another, depending on: (1) the raw material used (2) the particular process employed (3) the commercialization system and (4) the ultimate use of the product.

Fresh-filled pasta, particularly ravioli, are common products worldwide (Aureli *et al.* 1986). The dough is prepared by mixing and mechanically kneading wheat semolina, wheat flour, eggs, natural colors and preservatives and water. The filling is usually a mixture which may contain poultry, leafy vegetables, ricotta cheese, grated parmesan cheese, meat and spices. The manufacturing process for fresh pasta does not include a step ensuring the elimination of microorganisms. The primary sources of contamination are raw ingredients and contamination during processing. Both dough and filling are good substrates for microbial growth and toxin production, having values of a_w = 0.93 and pH = 5.5, typically (Rodriguez *et al.* 1991; Walsh *et al.* 1974). Heat-resistant toxins produced by *Bacillus cereus* and *Staphylococcus aureus* are not eliminated by pasta cooking, this being the main cause of the reported outbreaks attributed to pasta consumption. These foods have also been associated with outbreaks of salmonellosis (Bryant *et al.* 1992; Grady *et al.* 1986; Woolaway *et al.* 1986). In Argentina, *Salmonella spp.* was isolated from fresh spaghetti samples (Cortinez *et al.* 1988). Outbreaks of food-borne diseases due to *Salmonella enteritidis* in Argentina between 1986 and 1988 caused 239 registered episodes in which 210 strains were isolated from human feces and 59 from food. The main source of infection was related to raw eggs (Eiguer *et al.* 1990). These data show the pressing need for the application of HACCP in the manufacturing process of these products.

The objectives of the present investigation were (1) to analyze probable microbial hazards in the elaboration of ricotta-filled ravioli including household cooking and holding periods before serving, (2) to establish the critical control points of the whole process, and (3) to evaluate the shelf-life of the product based on the growth of total microbial counts during storage at chilling temperatures.

MATERIALS AND METHODS

An Italian-style fresh pasta manufacturing plant in La Plata region, Argentina, was visited and the process for the preparation of ricotta-filled ravioli

was studied stage by stage. The practices employed were studied in detail to identify sources and mechanisms of contamination. This included analysis of raw materials, partially-processed materials (dough and filling), final product, cooked product and drinking water used in the manufacturing process. Sampling plans were according with the local policy (Código Alimentario Argentino 1993).

Description of Food Product Preparation and Operations Involved

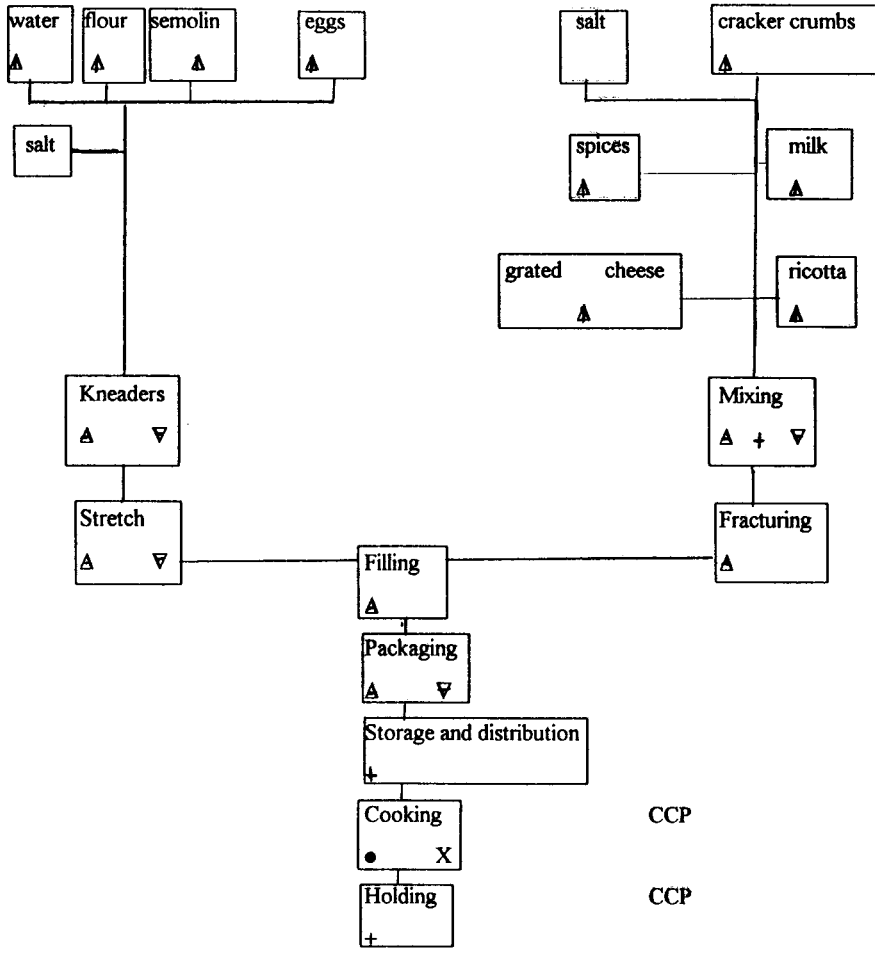
Among the different fresh pasta products made in the plant, a filled pasta (ricotta-filled ravioli) was selected due to the likely microbial contamination of its components. The ingredients and all processing stages are shown in Fig. 1. The raw materials used to prepare the dough were plain wheat flour 0000 (refined grade), wheat semolina, salt, fresh eggs and water. To obtain the filling, ricotta, spices (white pepper and nutmeg), salt, grated parmesan cheese, cracker crumbs and fresh whole milk were mixed at 25C. This filling was stored in a cold room (4-10C) for several hours until needed. Dough is filled mechanically, ravioli are formed by pressing with a grid-shaped matrix, then ravioli are sprinkled with wheat flour and packaged in carton boxes. The amount expected to be sold during the same day is stored at room temperature, while the remainder is chilled (4-10C). The industrial process finishes with the chilled storage. Samples of 50 g were cooked in 500 mL boiling water for 7 min to evaluate whether cooking time as recommended by the manufacturer was enough to produce a safe meal. Microbial growth and survival during holding after cooking, a common (household) practice, were evaluated after the cooked product was held overnight at room temperature.

Characteristics of the Plant

The small plant has three rooms, one for preparing the filling, another for dough and ravioli preparation and manual packaging, and the third one is the cold store. The cold room was used to store raw materials, partially processed material (filling) and packaged final product. Normally, ravioli production is 1300-1400 kg per month.

Hazard Analysis

The hazard analysis consisted of the following steps: (1) inspection of both preparation and storage practices to identify sources and modes of contamination (2) measuring temperatures of internal region of food during preparation, storage, cooking and holding (3) collecting samples in the preparation stages and testing them for microorganisms of concern and (4) collecting samples after cooking and after overnight holding to evaluate survival, destruction and growth of microorganisms and the decrease of risks associated with the operations.



▲ Hazard of contamination likely

Δ Hazard of equipment contamination

∇ Hazard of hand contamination

\+ Hazard of bacterial growth likely

● Hazard survival likely

X Microbial destruction likely

CCP Critical Control Point

FIG. 1. FLOW CHART FOR THE RICOTTA-FILLED RAVIOLI MAKING PROCESS

Samples of water used for food preparation were collected as well. Tested pathogens were selected on the basis of commonly occurring microorganisms found in the studied materials. Ingredients were sampled. Temperatures of the interior of food were recorded throughout preparation (including cooking and holding) by inserting thermocouples with needle-type sensors plugged into a Fluke 2240 C Data Logger. The pH was measured directly in the product using an insertion electrode (Ingold lot 405 m4, Urdof, Zurich, Switzerland). Moisture content was determined gravimetrically in duplicate by drying at 105C and atmospheric pressure until constant weight. Results were expressed as g of water per 100 g of initial sample. Water activity (a_w) was measured in a Novasina Thermoconstanter Humidat TH2/TH1 (Novasina, Zurich, Switzerland) that measures the equilibrium air relative humidity over the food sample in a small thermostatized sealed chamber. To calibrate the relative humidity (RH) sensor, its readings were previously adjusted in the measuring range by means of saturated solutions of K_2CrO_7, $BaCl_2.2H_2O$, NaCl and $MgNO_3.6H_2O$, with 98, 90, 75 and 52% RH, respectively, at 25C. Observations were made on likely sources of contamination and opportunities of cross contamination were considered as well. Microbiological counts of *Enterobacteriaceae, Bacillus cereus, Staphylococcus aureus,* molds and yeasts, sulfite-reducing *Clostridium* and total microbial counts were carried out. Critical control points of the operations were evaluated.

Microbial Determinations

Samples of approximately 200 g were aseptically collected at different points of the process and placed into sterile containers. They were immediately brought to the laboratory to assess microbial growth. Subsamples of 20 g were homogenized for 1 min with 180 mL 0.1% peptone water in a stomacher; 0.1 mL of the appropriate dilutions were inoculated on the following medias:

(1) Violet bile red glucose agar (Merck-Germany) was used for *Enterobacteriaceae* count. Plates were incubated at 37C for 12 to 24 h.
(2) Phenol red egg yolk polymixin agar (Mossel *et al.* 1967) (Merck) supplemented with 50,000 IU polymixin per liter and egg yolk emulsion was used for *Bacillus cereus* count. Plates were incubated at 35C for 2 days. Typical colonies were confirmed with starch, citrate, Voges-Proskauer and motility-nitrate test.
(3) Baird-Parker agar (Oxoid) supplemented with tellurite and egg yolk emulsion was used for enumeration of *Staphylococcus aureus.* Plates were incubated at 35C for 48 h. Typical colonies were transferred to BHI broth (Merck), incubated at 37C for 24 h and then examined by coagulase production (rabbit plasma EDTA, Difco).

(4) YGC agar (Yeast extract, glucose, chloramphenicol) (Merck) was used for enumeration of mold and yeast, with incubation at 25C for 5 days.
(5) Sulfite-reducing *Clostridium* counts were carried out using the most probable number (MPN) method. Test tubes with sulfadiazine polymyxin sulfite SPS (Merck) agar were inoculated with 1 mL dilution and were heated to 80C for 10 min. Tubes were incubated at 37C for 48 h in anaerobic condition. Gelatin-lactose and motility-nitrate tests were used for confirmation.
(6) Plate count agar (Merck) was inoculated with 1 mL dilution. Pour plate procedure was used for total microbial count with incubation at 30C for 2 days.

A colony counter (Inolex) was used, and results were expressed in CFU/g (colony forming units per gram) or Most Probable Number per g (MPN/g). All microbial tests were performed in triplicate; results were mean of six counts at three different dilutions.

The presence of the following microorganisms was also investigated: *Escherichia coli, Salmonella* spp. and *Pseudomonas fluorescens*. For *E. coli*, the AOAC Method 46016 (AOAC 1984) was used. For the detection of *Salmonella* spp, 25 g sample was added to a preenrichment medium with 225 mL lactose broth and was incubated at 37C for 24 h. One mL homogenate was inoculated to both tetrathionate and selenite broths; tubes were incubated at 37C for 48 h. Subcultures were transferred to bismuth sulfite agar and brilliant green phenol red lactose saccharose agar. Typical colonies were confirmed by serological testing using polyvalent antisera (Difco). *Pseudomonas fluorescens* was determined in samples of drinking water according to Standard Methods procedure, (APHA 1995).

RESULTS AND DISCUSSION

Application of HACCP Concept

The flow chart of the process is shown in Fig. 1. The diagram also indicates the hazards and critical points identified upon the analytical results obtained and on the inspection of the process. Table 1 shows the results obtained in the microbial analysis of raw materials, partially processed product, finished product, product after cooking and overnight holding as well as the pH of the samples.

Total microbial counts in ricotta, as raw material, were high (1.8×10^5 - 5×10^8 CFU/g) with mold and yeast counts 1×10^5 CFU/g. Moreover, both *Enterobacteriaceae* counts (5×10^3 - 1×10^5 CFU/g) and the presence of *E. coli* in one sample of five analyzed suggested that this raw material could be

potentially hazardous. The quality of ricotta depended to a great extent on the supplier, with pH values ranging from 6.2 to 4.5. In this regard, the samples with pH below 5.0 contained higher microbial counts (total values $> 1 \times 10^7$ CFU/g), which are responsible for the acid production. The quality of the incoming ingredients was critical to the counts in the final product. Therefore, establishment of prerequisite standards for incoming ingredients was recommended to the manufacturer.

TABLE 1.
MICROBIAL COUNTS (CFU/g) OF RAW MATERIALS, INTERMEDIATE AND FINAL PRODUCTS, COOKING PRODUCT AND AFTER HOLDING OVERNIGHT IN RICOTTA-FILLED RAVIOLI MANUFACTURING

Description	pH	Enterob.	E coli	Salm	B.c.	S.a.	Total	M/Y
flour	ND	$<10^2$	-	-	$<10^2$	$<10^2$	4.10^3	10^3
semolina	5.8	$<10^2$	-	-	$<10^2$	$<10^2$	$7\ 10^3$	410^2
spices	6.7	10^2	-	-	$<10^2$	$<10^2$	1.10^5	1.10^3
ricotta	6.2	$510^3 10^5$	+/-	-	$<10^2$	$<10^2$	10^5-10^8	10^5
dough	5.7	4.10^3	-	-	$<10^2$	$<10^2$	6.10^4	10^2-10^3
water	7.0						1.10^2	
ricotta-filling	5.4-5.8	10^5-10^6	+/-	-	$<10^2$	10^2	6.10^6	10^5
ricotta-filled ravioli	5.9	8.10^5	+/-	-	$<10^2$	$<10^2$	3.10^8	210^4
cooked product	5.8	$<10^2$	-	-	$<10^2$	$<10^2$	10^2	$<10^2$
after holding	5.8	$<10^2$	-	-	5.10^3	10^2	3.10^3	10^3

Total: Total microbial count CFU/g.
M/Y: Colony count of molds and yeasts CFU/g.
Salm: Presence (+) or absence (-) of *Salmonella sp.* in 25 g.
Enterob: Colony count of *Enterobacteriaceae/g.*
E. coli: Presence (+) or absence (-) of *E. coli* in 50 g.
S.a: Colony count of *S. aureus/g.*
B.c.: Colony counts of *Bacillus cereus/g.*

The grated parmesan cheese and pasteurized milk used to prepare the filling were not important sources of contamination, as indicated by the low counts of *Enterobacteriaceae* ($<10^2$ CFU/g) and the failure to detect *E. coli* in five samples analyzed.

In dough preparation, the use of fresh eggs could be dangerous, since they may contain *Salmonella* spp., particularly *S. enteritidis*. However in the present study, *Salmonella* spp was not detected in any of the five samples tested. The total microbial counts in the dough samples (pH 5.7) were 6.0×10^4 CFU/g with *Enterobacteriaceae* 4.0×10^3 CFUg; *E. coli* was not detected in the dough and the counts of molds and yeasts varied from 1×10^2 to 1×10^3 CFU/g for the dough and its constituents.

Water complied with local regulations for drinking water (total colony counts below 1×10^2 CFU/mL, coliforms at 44.5C below 2 MPN/100 mL, absence of *E. coli* and *Pseudomonas aeruginosa* in 100 mL water (Codigo Alimentario Argentino 1994)).

As previously found in ricotta as raw material, the ricotta filling had elevated total microbial counts (6×10^6 CFU/g) and *Enterobacteriaceae* (1×10^5-1×10^6 CFU/g). *E. coli* was detected in one sample of five tested samples. Sample pH varied appreciably (5.8-5.4) with the quality of the raw material. Counts of *B. cereus, S. aureus* and sulfite-reducing *Clostridium* were less than 10^2 CFU/g and <2MNP/g, respectively, in the studied materials.

In the finished product (ricotta-filled ravioli), total microbial counts and *Enterobacteriaceae* were very high, with average values of 3×10^8 and 8×10^5 CFU/g, respectively. The maximum levels specified for ricotta-filled ravioli in Argentina, CAA. Art. 720 (Codigo Alimentario Argentino 1994) are for: *S. aureus* positive coagulase n=5, c=2, m=10 /g and $M=1\times10^3$/ g; for *Salmonella* spp: n=5, c=0 and m=0; for sulfite-reducing *Clostridium* n=5, c=1, m=1 and $M=1\times10^3$ /g. In Argentina, Rodriguez *et al.* (1991) found total counts of 4.3×10^7 CFU/g, coliform and *E. coli* 1.1×10^6 MNP/g in ravioli samples. Pasolini *et al.* (1974) also reported high microbial counts in ravioli samples. Counts exceeded 10^9 CFU/g in 30% of the samples; 71% contained coliforms, and 46% contained *S. aureus*, while *Salmonella* spp. were not detected in any sample. The results of Spicher (1976) on microbial contamination of ravioli were of the same order. Therefore, our data and those obtained by other authors provide evidence that food hygienic procedures are often not correctly applied.

The water activity (a_w) of ricotta filling and ricotta-filled ravioli varied between 0.97 and 0.96 with a moisture content (wet basis) of 30-31%, making these foods a good substrate for microbial growth, including pathogens.

Consumer practices like cooking and holding foods after preparation were also evaluated since the industrial manufacturing process does not ensure the elimination of microorganisms. During cooking, ravioli reached the maximum

internal temperature (99.5C) in 5 min and were maintained in the boiling water bath for 2 more minutes. Immediately after cooking ricotta-filled ravioli had total microbial counts below 1×10^2 CFU/g, while pathogens were not detected. Cooked samples held overnight at room temperature (21C) reached total counts of 3×10^3 CFU/g, yeasts and molds reached 3×10^4 CFU/g and *Bacillus cereus* increased to 5×10^3 CFU/g. Neither *Salmonella* nor *E. coli* were isolated. *S. aureus* and sulfite reducing *Clostridium* showed counts below 1×10^2 CFU/g and 2MNP/g, respectively. While vegetative forms of pathogenic bacteria would have been killed during cooking, heat-resistant spores might have survived. Therefore, *B. cereus* and sulfite reducing *Clostridium*, spore-forming pathogens, are of primary concern.

After the analysis, critical control points for elaboration of ricotta-filled ravioli were identified as (1) cooking and (2) holding time before serving. Cooking can be monitored by observing that the food remains for 7 min in boiling water. Monitoring of holding period can be done by observing that the food is eaten promptly after cooking or the duration of holding time.

Improvements of Manufacturing Process

From the present investigation some drawbacks concerning sanitary conditions were corrected. At the end of the working day, both the mixer and the kneader were thoroughly washed with a solution of sodium hypochlorite (0.02% active chlorine) for 30 min and then rinsed several times with drinking water. Another action taken was the incorporation of basins with liquid soap allowing periodic hand-washing of operators.

Both the machine used to rub and stretch the dough and that used to form the ravioli were dry-cleaned by high power vacuum cleaners to remove dry residues. Periodically, additional swabbing of the equipment has been done.

In addition, the use of separate cold stores for raw materials and final product to avoid cross-contamination was suggested. It was recommended that products made first should be removed first from the store, i.e. "first in first out".

After all corrections suggested were implemented, the process was inspected again, and the corresponding results are shown in Table 2. Considerable improvements were obtained in microbial quality of the finished product, owing to the use of raw materials of better quality and to the adopted corrections regarding process hygienics. People in Argentina are routinely buying pasta with more than 1×10^8 CFU/g with no substantial health problems. But, it should be remembered that these products are always eaten cooked; people boiled fresh pasta in water for more than 7 min. Thus, the Argentinean pasta industry is qualified to produce better quality products, provided good manufacturing

practices, critical control points analysis and raw materials of adequate quality are considered.

TABLE 2.
MICROBIAL COUNTS (CFU/g) IN RAW MATERIALS, PARTIALLY-PROCESSED PRODUCTS AND FINAL PRODUCTS AFTER APPLYING SUGGESTED CORRECTIVE ACTIONS*

Description	pH	Enterob	E. coli	Salm.	B.c.	S.a.	Total	M/Y
dough	5.8	$<10^2$	-	-	$<10^2$	$<10^2$	2.10^4	10^3
ricotta	6.4	1.10^3	-	-	$<10^2$	$<10^2$	4.10^4	4.10^2
ricotta-filling	6.3	5.10^4	-	-	$<10^2$	$<10^2$	3.10^5	7.10^4
ricotta-filled ravioli	5.9	9.10^4	-	-	$<10^2$	$<10^2$	5.10^5	8.10^4

* For abbreviations, see legend of Table 1

Prediction of Product Shelf-life

Generally, HACCP analysis is often limited to the manufacturing plant, with the distribution chain of finished product receiving less attention. Fields (1989) has reported that distribution often presents considerable temperature variations, reducing product shelf-life and increasing the risk of disease transmission.

To predict product shelf-life, we followed total microbial growth at different temperatures after recommended suggestions were implemented. Figures 2 a and b show the average growth of total microbial counts in ricotta and in ravioli, respectively, during storage at 0, 4, 8 and 10C. Ricotta initial counts are one logarithm cycle lower than those of ravioli. For ricotta, microbial growth showed a marked temperature dependence; at 8 and 10C microbial growth rates were twice those obtained at 0 and 4C. Thus, storage temperature of ricotta should be strictly controlled. Microbial growth rates of ravioli were similar for the four temperatures studied. Shelf-life of the ricotta and ricotta-filled ravioli can be defined as the time of chilled storage at which the product

reaches microbial counts of 1×10^6 CFU/g. According to Howard and Dewi (1995) toxic substances may be produced when microbial counts exceed 1×10^6 CFU/g. The predicted shelf-life of ricotta varied from 16.8 to 56.4 h in the covered temperature range (0 to 10C), while ricotta-filled ravioli had shorter shelf-life periods ranging from 1.2 to 14.0 h (Table 3). This analysis allows the calculation of the shelf-life of the product as a function of storage temperature, a necessity for consumer instructions.

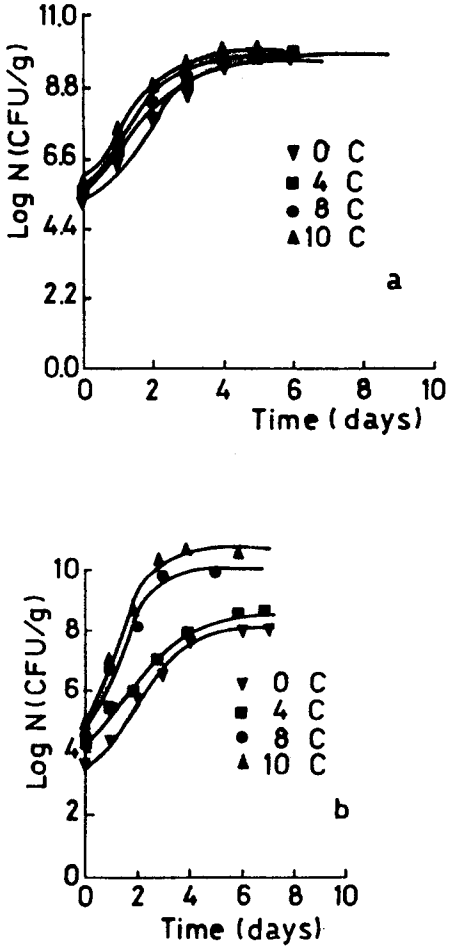


FIG. 2. MESOPHILIC COLONY COUNT AT DIFFERENT STORAGE TEMPERATURES IN (a) RICOTTA SAMPLES AND (b) RICOTTA FILLED RAVIOLI SAMPLES

TABLE 3.
SHELF-LIFE PERIODS IN HOURS FOR RICOTTA AND RICOTTA-FILLED RAVIOLI SAMPLES STORED AT DIFFERENT TEMPERATURES

Temperature (°C)	Ricotta samples time to reach 10^6CFU/g	Ricotta-filled ravioli samples time to reach 10^6 CFU/g
0	56.4	14.0
4	55.2	7.2
8	30.7	6.7
10	16.8	1.2

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