MICROBIAL ENUMERATION IN READY-TO-EAT FOODS AND THEIR RELATIONSHIP TO GOOD MANUFACTURING PRACTICE

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ABSTRACT

As a contribution to Good Manufacturing Practice (GMP) programs, we have analyzed products produced and offered by ready-to-eat food stores. Two hundred and seventy four samples of a wide variety of foods from 19 different shops were analyzed. Aerobic counts, total coliforms and yeast and molds were enumerated in each sample. Food stores were evaluated using a GMP check-list. They were grouped in three classes: Class III, nonsatisfactory GMP; Class II, partially satisfactory GMP; and Class I, satisfactory GMP. From the results of microorganism counts in the Class I food shops the following maximum counts are proposed for cooked ready-to-eat foods: aerobic colony count: 10^5 cfu g^{-1}. It is coincident with satisfactory microbiological quality of many category 3 foods drawn up by Public Health Laboratory Service (PHLS).

INTRODUCTION

The marked increase in the number of ready-to-eat food stores in Argentina during the last few years has presented the health control authorities with a new surveillance task. Contamination of ready-to-eat foods with spoilage microorganisms and food-borne pathogens has been recognized long ago. Albrecht *et al.* (1995) analyzed aerobic plate counts, total coliforms and yeast and molds in vegetables from salad bars and found contamination and/or growth of existing microflora.

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Beuchat (1995) detected pathogenic microorganisms associated to gastroenteritis in raw vegetables and fresh fruits. O'Connor-Shaw *et al.* (1994) studied the significance and control of coliforms in processed mango. They found *K. pneumoniae* as the predominant coliform. The presence of *K. pneumoniae* is considered undesirable in view of its opportunistic pathogenic nature. Furlanetto *et al.* (1982) found increased number of microorganisms in salads and salad dressings at different restaurants and food shops. Tamagnini and González (1997) determined aerobic colony count, coliforms, *E. coli* and *Pseudomonas aeruginosa* in bottled water. *P. aeruginosa* was isolated from commercial products bottled in reused plastic containers probably due to lack hygiene practice fulfillment at factory.

Good Manufacturing Practice (GMP) and Hazard Analysis Critical Control Point (HACCP) programs are being developed to minimize the risk of illness associated with food consumption. HACCP control procedures are directed at specific critical control points (CCP) at which a hazard can be eliminated or reduced to acceptable levels. Information on the probable microbial load at time of consumption, probability of survival of organisms during processing and impact of abuse conditions are necessary to define acceptable levels on a CCP (Notermans *et al.* 1995). Other authors agree that microbiological testing is necessary for the implementation and maintenance of effective HACCP systems for assuring the safety of foods and that microbiological testing must involve the enumeration of indicator organisms rather than the detection of pathogens (Brown *et al.* 2000; Swanson and Anderson 2000). Majewski (1992) proposed that, although HACCP is most readily applied to manufacturing processes, attempts are being made to adapt the system to other sectors of the food industry, such as catering.

The purpose of this paper is to discuss the relationship between the aerobic colony counts (ACC), total coliforms (TC) and yeast and molds (YM) with Good Manufacturing Practice (GMP) fulfillment in ready-to-eat food stores.

MATERIAL AND METHODS

Food Samples

Two hundred and seventy four (274) samples of a wide variety of ready-to-eat foods offered in different food shops (sandwich bars, cafes, delicatessens and restaurants) and prepared on site, were analyzed (Table 1). They were obtained in 19 food shops from Córdoba, Argentina along a two-year period (January 1998-December 1999).

TABLE 1.
FOOD SAMPLES

Food	Sample	Description
Bechamel	7	Prepared with milk, flour, butter and nutmeg
Boiled vegetables salad	40	Potato, carrot, green peas with mayonnaise dressing
Cooked vegetables	51	Chard with bechamel, lentils, potato, eggplant, vegetable pie, cabbage, etc.
Eggs	10	Sliced hard-boiled
Fish	4	Hake, shellfish and octopus
Fruit salad	4	With apple, orange, pineapple, banana, etc
Meat and meat meals	28	Meatballs; meat pie; roasted meat; sliced boiled meat served cold with tuna fish, anchovy and mayonnaise sauce ("vittel tonnee"), etc.
Pasta	23	Spaghetti, cappelletti, ravioli, etc.
Poultry	29	Poultry with salads, cooked vegetables, stuffed-chicken, etc.
Rice	31	White; with saffron; mixed with salads, cooked vegetables, ham or poultry, etc.
Salads	30	Lettuce, tomato, cabbage, beet, carrot, with or without mayonnaise, etc.
Sandwiches	17	White bread with jam, cheese, vegetables, etc.

Evaluation of Good Manufacturing Practice (GMP)

Ready-to-eat food shops were evaluated using a GMP check-list that included: personnel uniform, handling, working surfaces, sanitization practices and refrigerators. In sanitization practice item, "defined procedure" and "execution" subitems were classified as fulfilled (2) or not fulfilled (1). The rest of sub-items were rated as nonsatisfactory (1), partially satisfactory (2) and satisfactory (3). The value for each item was obtained multiplying by the weight unit (Table 2). According to the sum total obtained for each shop, they were grouped into three classes: Class III, nonsatisfactory GMP (23 to 35); Class II, partially satisfactory GMP (36 to 49); and Class I, satisfactory GMP (50 to 66).

TABLE 2.
CHECK-LIST OF GOOD MANUFACTURING PRACTICE

Items	Subitems	Weight Unit
Personal uniform	Hygienic conditions	1
	Use	1
Handling		4
Working surfaces	Materials	2
	Cleanness	4
Sanitization practice	Defined procedure	1
	Effectiveness	3
	Execution	2
Refrigerator	Cleanness	2
	Temperature	3

Sample Preparation

Approximately 100 g of each food type was collected using sterile utensils and the samples were placed into sterile Whirl-Pak Bags (Nasco). The samples were immediately transported to the laboratory in an icebox (2-6C). The microbiological analysis was initiated 1 to 3 h after sampling. A 10 g analytical unit of each food was homogenized with 90 mL of sterile water-peptone (0.1%) for 2 min and then serial 10-fold dilutions were prepared with sterile water-peptone (Anon. 1992).

Aerobic Colony Count Method (ACC)

Duplicate 1 mL pour plates using Plate Count Agar (PCA) were prepared using dilutions of each analytical unit and incubated at 29-31C for 3 days. The plates that did not develop colonies were computed as count 1 colony forming unit (cfu) multiplied by the smallest dilution factor used (Anon. 1992). The higher limit of detection was 10^7 cfu g^{-1} and the lower was 2×10^3 cfu g^{-1}.

Total Coliform Colony Count Method (TC)

Duplicate 1 mL pour plates with a Violet Red Bile Agar (VRBA) overlay were prepared using dilutions of each analytical unit. Plates were incubated at

35-37C for 24-48 h. The plates that did not develop colonies were computed as count 1 colony forming unit (cfu) multiplied by the smallest used dilution factor (Anon. 1992). The higher limit of detection was 1×10^4 cfu g^{-1} and the lower was 10 cfu g^{-1}.

Yeast and Molds Colony Count Method (YM)

Duplicate 0.2 mL spread plates using Mycophilic agar (MYC) were prepared using dilution of each analytical unit and incubated at 22-25C for 5 days. The plates that did not develop colonies were computed as count 1 colony forming unit (cfu) multiplied by the smallest used dilution factor (Anon. 1992). The higher limit of detection was 25×10^3 cfu g^{-1} and the lower was 25 cfu g^{-1}.

Statistical Analysis

The microorganism counts obtained were grouped as follows:(1) foods from different shop classes, (2) foods with or without handling after cooking, (3) cooked or raw foods, (4) predominant ingredients, (5) foods from different seasons.

Colony counts were converted to $\log_{10}$ counts $\times$ g^{-1}. Mann-Whitney Rank Sum test and Kruskall-Wallis test were applied. All statistical methods were performed with SigmaStat 2.03.

RESULTS AND DISCUSSION

Evaluation of Good Manufacturing Practice

According to the GMP value, 5 ready-to-eat food shops were classified as Class I (satisfactory GMP), 7 shops as Class II (partially satisfactory GMP) and 7 shops as Class III (nonsatisfactory GMP).

The most frequent unsatisfactory practices found in the shops were: unsanitized or partially sanitized kitchenware, working surfaces and tablecloths; contact of raw and cooked foods during processing; foods prepared 24 h or longer before consumption; in some cases, time of food preparation was unknown; unstandardized time of cooling for cooked foods; inadequate temperature in refrigerators; storage of cooked and raw foods in the same refrigerator compartment; foods stored and piled up in containers without cover; expired perishable goods; personnel not wearing hair nets or showing unclean clothes or fingernails.

Microorganism Counts Related to GMP Classes

Table 3 shows the relationship between microorganism counts and GMP shop classification. Aerobic plate counts in Class I shops were significantly smaller than those in Class II and III ($P < 0.05$); yeast and molds were higher in Class III shops (3.451 log cfu g^{-1}) ($P < 0.05$). The total coliform counts were different in the three shop classes ($P < 0.001$). The median increased from 1.00 log cfu g^{-1} for Class I, to 2.18 for Class II to 3.30 for Class III. These results indicated that the hygiene parameters used for classification GMP fulfillments were correct. Coliforms have been traditionally used as a pathogen indicator in foods (de Boer 1998). Mosupye and von Holy (1999) found Enterobacteriaceae counts value of 2 log cfu/g in ready-to-eat street food samples. These data are coincident with total coliform counts found in foods of Class II shops. Furthermore, they detected *Bacillus cereus*, *Clostridium perfringens* and *Salmonella* spp in foods.

TABLE 3.
MICROORGANISM COUNTS IN FOODS FROM SHOPS OF DIFFERENT CLASSES

Shop Class	Sample	Aerobic Colony (log cfu/g). Median	Yeast and Molds (log cfu/g). Median	Total Coliforms (log cfu/g). Median
I	70	4.482*	2.000*	1.000 *
II	85	4.778 †	2.477*	2.182†
III	136	5.301 †	3.451†	3.301‡
		$P < 0.001$§	$P < 0.001$ §	$P < 0.001$ §

*,†,‡ Significant difference: $P < 0.05$, § Significant difference: $P < 0.001$.

Aerobic plate counts and yeast and molds represent an unreliable and inexact measure of food contamination (Anon. 1982). In agreement with these previous studies, our results indicate that coliforms are the best indicator of GMP fulfillment.

On the basis of microorganism counts in foods from Class I shops the following guideline for cooked ready-to-eat foods appear reasonable: aerobic colony counts: 10^5 cfu g^{-1}. It agrees with the satisfactory microbiological quality of many category 3 foods (sliced meat, cooked fish, rice, vegetables, etc.) drawn up by Public Health Laboratory Service (PHLS) (Gilbert 1996).

Microorganism Counts in Foods With or Without Handling

Foods with or without handling after cooking did not show significant differences of microorganism counts in Class I shops ($P > 0.1$) (Table 4). In Class II and III shops microorganism counts were higher in foods with handling after cooking compared to those unhandled ($P < 0.05$) (Table 4). Personnel in Class II and III shops used partially or not sanitized kitchenware and working surfaces, favoring cross contamination. Beuchat (1995) stated that hygienic practices of workers and adequate sanitization in processing environments can easily lead to a reduction of safety microbiological risks. Josephson *et al.* (1997) evaluated the prevalence of indicator bacteria and specific pathogens in kitchen with and without use of disinfectant cleaner. These data showed that normal kitchens can easily be contaminated with a variety of bacterial including *Salmonella* and *Campylobacter*. They concluded irregular use, or not using antimicrobial agents, is unlikely to reduce the risk of these infectious agents.

TABLE 4.
MICROORGANISM COUNTS IN FOODS WITH OR WITHOUT HANDLING

	Handling	Sample	Aerobic Colony (log cfu/g) Median	Yeast and Molds (log cfu/g) Median	Total Coliforms (log cfu/g) Median
CLASS I shops	With	32	4.82	1.94	2.27
	Without	14	4.56	1.40	1.00
			P =0.924	P =0.417	P =0.181
CLASS II shops	With	45	5.59	2.85	3.13
	Without	10	3.30	2.00	1.00
			P =0.005*	P =0.047*	P <0.0001*
CLASS III shops	With	94	5.41	3.52	3.81
	Without	31	4.59	2.70	2.67
			P =0.026*	P =0.0043*	P <0.0001†

* Significant difference: P<0.05, † Significant difference: P<0.0001.

Microorganism Counts in Cooked and Raw Foods

This analysis was not performed in Class I shops due to the small number of samples. No significant differences were found in the aerobic colony counts and total coliforms in cooked and raw foods revealing the lack of GMP application.

The high bacterial count in cooked foods would indicate that they were contaminated during after-cooking handling procedures, demonstrating an overall lack of hygiene. Other studies have shown that during the preparation of raw meat and vegetables in kitchens, numerous surfaces can become contaminated, the contaminating microorganisms can survive for considerable periods of time (Scott and Bloomfield 1990; Gillespie *et al.* 2000). In these instances, the cross-contamination may occur. Significant differences between raw and cooked foods were only observed in yeast and molds in Class II and III shops (Table 5).

TABLE 5.
MICROORGANISM COUNTS IN RAW AND COOKED FOODS

	Foods	Sample	Aerobic Colony (log cfu/g) Median	Yeast and Molds (log cfu/g) Median	Total Coliforms (log cfu/g) Median
CLASS II shops	Cooked	58	4.79	2.44	2.70
	Raw	16	4.95	4.05	3.50
			P =0.604	P <0.0001†	P =0.111
CLASS III shops	Cooked	121	5.30	3.43	3.51
	Raw	16	5.48	3.91	4.02
			P =0.507	P =0.010*	P =0.475

* Significant difference: P<0.05, † Significant difference: P<0.001.

The mean value of aerobic colony counts in raw foods was similar to that reported by Albrecht *et al.* (1995) in lettuce and tomato (5.70 and 5.51 log cfu g^{-1}, respectively); these authors inferred an excessive handling or crossed contamination during the processing. Our values for yeast and molds and total coliforms were lower than those found by Albrecht *et al.* (1995) (total coliforms varied between 5.4 and 5.2 log cfu g^{-1}; yeast and molds between 6.26 and 6.78 log cfu g^{-1} in tomato and lettuce). In Class II and III shops fruits and vegetables were not sanitized. Fruits and vegetables usually contain populations of 10^4 to

10^6 microorganisms g^{-1} when they arrive in the shops. The usual washing procedure does not completely remove microorganisms: sanitation is needed to significantly reduce microbial populations (Beuchat 1995).

Microorganism Counts in Different Foods

The analysis of the microbial counts in different foods were performed independently of the shop Classes (Table 6). No significant differences in aerobic colony counts were found among the foods ($P = 0.059$), the medians varied from 3.30 to 5.70 log cfu g^{-1} due to wide dispersion of the values. Our results show that 25% of the ready-to-eat foods analyzed (except for sandwiches and salads) exceeded aerobic colony counts (ACC) guideline: > 5 log cfu g^{-1}. Salads and sandwiches did not exceed the ACC guideline expected for these products: > 7 log cfu g^{-1} (Gilbert 1996).

TABLE 6.
MICROORGANISM COUNTS IN DIFFERENT FOODS

Foods	Sample	Aerobic Colony (log cfu/g) Median	Yeast and Molds (log cfu/g) Median	Total Coliforms (log cfu/g) Median
Rice	31	5.34	3.41	3.30
Meat	28	4.98	3.16	2.73
Salads	30	5.38	3.91	3.80
Boiled vegetables salad	40	4.86	3.13	3.73
Eggs	10	4.25	1.87	1.94
Pasta	23	5.51	3.69	3.59
Poultry	29	5.41	2.40	2.54
Bechamel	7	3.30	1.40	1.00
Sandwiches	17	5.70	3.93	2.82
Cooked vegetables	52	4.82	2.37	2.99
		$P=0.059$	$P<0.0001$*	$P<0.015$ *

* Significant difference: $P<0.05$.

Salads and sandwiches had the highest yeast and molds (YM) counts (median 3.91 and 3.93 log cfu g^{-1}, respectively) while hard-boiled eggs and bechamel had the lowest counts (1.87 and 1.40 log cfu g^{-1}, respectively). The medians of total coliforms (TC) ranged from 3.80 log cfu g^{-1} in salads to 1.0 log cfu g^{-1} in bechamel. These results would indicate that food with raw ingredients and handled-after-cooking foods had significant higher counts. Cooked vegetables showed high dispersion of TC; the 50% of the samples varied between 1.00 and 4.36 log cfu g^{-1}. These results could be explained by the inclusion of foods with or without manipulation after cooking in the same group; the high TC could be the result of nonhygienic handling.

Magri *et al.* (1996) found a wide range of ACC when analyzing pasta with and without filling. In pasta with filling, 50% of the samples varied from 0 to 3×10^6 cfu g^{-1} with a median around 25×10^4. Counts of total coliform varied from 0 to 1×10^4; these values were similar to the results obtained in this study, where ACC medians were 5.51 log cfu g^{-1} and 50% of TC varied between 2.98 and 4.75 log cfu g^{-1}.

Furlanetto *et al.* (1982) found ACC in salad dressings from 2.64×10^4 cfu g^{-1}; yeast and molds varied from 710 to 3.7×10^6 cfu g^{-1} and for TC the range was between 0 and 4.3×10^6 cfu g^{-1}. Our results in 50% of the counts in boiled vegetables salad are coincident with the values obtained by those authors, who found pathogenic bacteria that resist the acidity of the salad.

Microorganism Counts in Different Seasons

The comparison among the counts of the three groups of microorganisms showed no significant differences among seasons (Table 7). The aerobic colony counts medians varied between 4.90 and 5.30 log cfu g^{-1}, yeast and molds between 2.88 and 3.48 log cfu g^{-1}, and total coliforms between 2.83 and 3.30 log cfu g^{-1}. The temperature in the kitchens is unrelated to external temperature

TABLE 7.
MICROORGANISM COUNTS AMONG SEASONS

Season	Sample	Aerobic Colony (log cfu/g) Median	Yeast and Molds (log cfu/g) Median	Total Coliforms (log cfu/g) Median
Summer	53	4.90	3.48	2.83
Autumn	76	5.00	3.25	3.06
Winter	84	5.16	2.88	2.87
Spring	62	5.30	2.95	3.30
		P=0.784*	P=0.142*	P=0.614*

* No significant difference: $P>0.05$

because of factors such as: the vapor from the cooking process, heat generated by ovens, burners and refrigerator equipments, poor air extraction, etc. These factors increase the humidity and temperature indoors independent of external conditions.

CONCLUSION

The high microbial counts in ready-to-eat foods of Class II and III shops, show that contamination during handling, inadequate or nonexistent sanitation, contaminated raw vegetables, long food storage, high indoors temperature and humidity or a combination of these factors was involved. This may indicated a deficiency in management training, resulting in less stringent food hygiene procedures and a lower standard of microbiological quality of the foods provided.

The enumeration of indicator microorganisms could be applied to defined acceptable levels on critical control point in the ready-to-eat foods production.

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