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Short communication

Non-lactic acid, contaminating microbial flora in ready-to-eat foods: A potential food-quality index

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Abstract

The bacteriological profile of 87 samples of commercially available ready-to-eat (RTE) dairy and meat-products, packaged sandwiches and salads was obtained by testing for aerobic colony count, for lactic acid bacterial (LAB) count, for the presence and the extent of non-LAB microflora (contaminating microflora), and by testing for certain food-borne pathogens. The pathogens *Listeria monocytogenes, Salmonella* spp. and sulfite-reducing clostridia were not detected in any of the analysed samples. Whereas only three samples (3.4%) were deemed unacceptable for consumption for exceeding the established pathogen tolerance levels (for *Staphylococcus aureu*s and *Escherichia coli*), several samples were found to contain non-lactic acid contaminating microflora of considerable magnitude. The $\log_{10}$ cfu g^{-1} counts for contaminating microflora in the food categories examined were as follows: hard cheeses 4.85 (SD 1.17); semi-hard cheeses 5.39 (SD 1.37); soft cheeses 5.13 (SD 1.03); whey cheeses 6.55 (1.24); fermented meat-products 4.18 (SD 1.48); heat-treated meat-products 3.47 (SD 1.99); salads 3.37 (SD 1.56) and sandwiches 5.04 (SD 0.96). Approximately 1 in every 30 to 80 bacterial cells found on different types of cheeses and salads was a non-LAB microorganism; the respective ratios for fermented meat-products, heat-treated meat-products and sandwiches were 1 in 6, 2.5 and 15. The assessment of the contaminating microflora magnitude at various steps during the manufacture and distribution of RTE foods can serve as an index for monitoring the microbiological quality of the starting materials, the sanitation efficacy during processing and possible temperature abuse during processing, transportation or storage.

Keywords: Ready-to-eat foods (RTE); Contaminating flora; Microbiological quality

1. Introduction

Consumers demand a wholesome and safe food supply. The modern way of life relies heavily on the availability, quality and safety of ready-to eat (RTE) foods. Whereas the quality of the starting materials is always of major importance, factors such as handling, processing, transportation and storage can influence the microbiological composition of the finished product at the consumer's table. Consequently, several investigators worldwide have examined the microbiological quality of their regional RTE foods (Yamani and Al-Dababseh, 1994; Kaneko et al., 1999; Gillespie et al., 2000; Johannessen et al., 2002; Fang et al., 2003).

Studies on the microbiological quality of foods employ established analytical protocols to assay for indicator and pathogenic organisms and then classify

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the foods according to legislatively established microbiological limits, criteria and proposed guidelines (Kaneko et al., 1999; Gillespie et al., 2000; Thunberg et al., 2002; Fang et al., 2003; Richardson and Stevens, 2003). Commonly adopted indices of microbial quality include the coliforms, the Enterobacteriaceae and the aerobic plate count (APC) (Pierson and Smoot, 2001). The APC criterion, being of general nature, yields no information about the identity of the constituent food microflora and therefore is of limited interpretational value. Moreover, this restraint renders it non-meaningful in cases where the microbial quality of fermented products, that are rich in lactic acid bacteria (LAB), is under debate.

The purpose of this study was to obtain the bacteriological profile of a broad spectrum of commercially available RTE foods at the point of sale in Greece. To our knowledge, this is the first report of this kind regarding national RTE products. In particular, we wanted to obtain an estimate about the presence and the magnitude of the contaminating microflora in these products. To achieve this goal, the subset of contaminating microflora was selectively enumerated and compared to the levels of fermentative microorganisms in several food categories. The RTE foods in this study were classified according to their basic constituents and technology of manufacture and underwent three distinct microbiological evaluations: APC, LAB count and non-LAB (contaminating microflora) count. The sampled food items were also analysed for coliform counts and for the presence and/or the level of certain food-borne pathogens as dictated by Hellenic Military regulations (Hellenic Presidential Decree (HPD) 9/1989, 1989; Council of the European Communities (CEC), 1992; HPD 56/1995, 1995; Hellenic Army General Staff (HAGS), 2001). In this communication, we show that the count agar sugar free (CASF) medium is suitable for enumeration of the non-LAB contaminating microflora of fermented (and non-fermented) foods and that, using this medium, an 'APC' can be obtained for fermented foods.

2. Materials and methods

2.1. Sampling

Eighty-seven food items originating from a wide range of national manufacturers were collected from different army-supermarkets in the northeastern counties of Evros, Rodopi and Xanthi in Greece. Among the 87 samples, 42 were cheese samples, 10 of which belonged to the hard cheese category (e.g. Kefalotyri, Graviera), 14 to the semi-hard cheese category (e.g. Makedoniko, Thrakiotiko, Kaseri), 12 to the soft cheese (e.g. Feta, Telemes), and 6 to the whey cheese category (Myzithra, Anthotyros, Manouri). Twenty-six were samples of meat-products [11 samples of fermented meat-products (salami) and 15 samples of heat-treated meat-products], 7 were RTE packaged salads (tzatziki, eggplant-salad, gardener's salad and Russian salad), and 12 were packaged sandwich items containing sliced cheese, sliced meat-product and salad spread. Trained veterinary officers conducted the sampling as part of a Hellenic Army regular monitoring program conducted by the Food and Water-Testing Laboratory (FWTL) of the Detachment of Veterinary Support of the 492-Military Hospital. On any given sampling date, sampled food items were transported on ice to the laboratory within a maximum of 3 h after sampling, stored at 4 °C overnight, and subsequently subjected to bacteriological analyses.

2.2. Bacteriological analyses

2.2.1. General bacteriological profile

Each food was analysed for APC (HAGS, 2002h), LAB and contaminating microflora. Twenty-five gram portions were aseptically removed from the package, mixed with 225 ml of cold sterile Buffered Peptone-Water (BPW) (Merck, Darmstadt, Germany) in sterile stomacher bags (ca. 400 ml capacity) with filter inlays in a Masticator Stomacher (IUL Instruments, Barcelona, Spain) for 90 s. Appropriate decimally diluted volumes in sterile Maximum Recovery Diluent (MRD) (Merck) were then pour-plated in triplicate in Plate Count Agar (PCA) (Merck), All Purpose medium with Tween® (APT) agar plates (Merck) containing 0.01% cyclohexamide, and Count Agar Sugar-Free (CASF) agar plates (Merck). APT plates were overlaid with ca. 7 ml of APT agar after solidification. All plates were left for 2 h at room temperature and then incubated for 48 h at 35 °C. CASF plates were subsequently incubated for an additional 48 h at 20 °C. For each food, 20 colonies from APT plates of the highest countable dilution (25–250 colonies per plate) were checked for gram and catalase reaction using appropriate controls. Uninoculated plates of all three kinds of media as well as plates inoculated with MRD served as controls.

2.2.2. Pathogen-testing

Like all foods that are routinely sampled by the FWTL, the 87 foods in this study were analysed for coliforms and for the following pathogenic microorganisms according to Hellenic Army requirements, which in turn are based on National and European Community legislation (HPD 9/1989, 1989; CEC, 1992; HPD 56/1995, 1995; HAGS, 2001): *E. coli*, *L. monocytogenes*, *Salmonella* spp., *S. aureus* and sulfite-reducing clostridia. The testing implementation and the specified tolerance levels (e.g. for coliforms and *E. coli*) vary depending on the food category under consideration,

whereas other tests are mandatory with uniform tolerance levels for all types of foods in this study (e.g. testing for *Salmonella* spp. and *L. monocytogenes* is mandatory in all foods with the requirement of absence of both organisms in 25 g of food). The analyses were conducted using ISO-based analytical protocols established by the Hellenic Army for the microbiological examination of foods (HAGS, 2002a–h).

3. Results and discussion

3.1. Bacteriological profile of RTE foods

Whereas the main objective of this work was to measure the levels of contaminating microflora in RTE foods, an estimate of its magnitude relative to the levels of LAB and total bacterial flora in these foods was also desirable. Therefore, dilutions of each food were plated on three general-profiling media, PCA, APT and CASF. Table 1 presents a general classification of the foods examined in this study along with the average microbial counts obtained on each of the three media used. PCA is a non-selective complex medium commonly used for enumerating the total microbial content in foods and water. APT is a complex medium designed for the culture and enumeration of LAB; it is rich in glucose (1%) and contains thiamine, a growth factor for LAB. All sets of APT colonies checked, as described in Materials and Methods, consistently turned out to be gram-positive and catalase-negative. Finally, CASF is a medium designed to detect the so-called 'infective (non-indigenous) microorganisms, i.e. those organisms which are not directly involved in the microbiological production of a food product, or which do not belong to its specific flora' (International Dairy Federation (IDF), 1991). It contains peptone, sodium chloride and agar only, and, being a sugar-free medium, cannot support the growth of LAB that are strictly fermentative organisms. The CASF counts can therefore be regarded as a biochemically selective bacteriological index. Since LAB do not grow on CASF, and given that LAB is the only microbial group that should be present in foods (added intentionally in the case of fermented foods), the presence in RTE foods of any other microorganism that grows on CASF is unintentional, and it is therefore justifiable to refer to such microbes as non-LAB or contaminants. These microorganisms may belong to heterogeneous bacterial genera and are capable of growing using peptides and amino acids as a sole carbon and energy source, whether they possess additional biochemical pathways or not.

Owing to their manufacturing technology, the microflora of successfully ripened milk-products and fermented meats should be mainly composed of LAB; indeed, this has been demonstrated for all groups of fermentation products in this study as shown by their cfu counts on APT relative to their counts on PCA (Table 1). It should be noted that a PCA count might not always constitute a guaranteed and unconditional numerical representation of a food's bacterial entirety. The percentage of viable bacteria in a food that will turn up culturable on PCA may depend on the nature of the food being analysed (the growth characteristics of the food's predominant microbial flora and the plethora of physiologically different microbial genera present), the medium's mode of use (spread vs pour plates) and the chosen combination of time/temperature of incubation. Also, it is not possible to document what fraction of colonies on a PCA plate consists of LAB vs non-LAB. However, it is noteworthy that in almost all food categories tested in our experiments, the sum of the average cfu counts on CASF and on APT agar plates approximately equaled the average number of cfu counts on PCA. This may be indicating that, at least under the particular experimental setting, counts on CASF and APT represent two distinguishable bacterial populations that essentially comprise the total mesophilic flora in these food categories.

Table 1
Log_{10} cfu g^{-1} counts (average ± SD) of different categories and types of ready-to-eat foods on three different media (CASF, count agar sugar-free; APT, all purpose medium with Tween®; PCA, plate count agar)

Category	Type	CASF	APT	PCA	CASF + APT[a]
Cheeses	Hard ($n = 10$)	4.85 ± 1.17	5.99 ± 1.39	6.36 ± 1.09	6.04 ± 1.36
	Semi-hard ($n = 14$)	5.39 ± 1.37	7.14 ± 0.82	7.24 ± 0.66	7.17 ± 0.82
	Soft ($n = 12$)	5.13 ± 1.03	6.67 ± 0.98	7.02 ± 0.59	6.69 ± 0.98
	Whey ($n = 6$)	6.55 ± 1.24	8.13 ± 0.57	8.19 ± 0.49	8.17 ± 0.53
Meat products	Fermented ($n = 11$)	4.18 ± 1.48	4.69 ± 1.92	4.94 ± 1.78	4.92 ± 1.72
	Heat-treated ($n = 15$)	3.47 ± 1.99	3.06 ± 2.15	3.85 ± 1.80	3.78 ± 1.91
	(Heat-treated)[b] ($n = 13$)[b]	(2.86 ± 1.27)[b]	(2.36 ± 1.23)[b]	(3.28 ± 1.03)[b]	(3.16 ± 1.06)[b]
Various salads	($n = 7$)	3.37 ± 1.56	4.87 ± 1.91	5.11 ± 2.02	4.91 ± 1.89
Sandwiches	($n = 12$)	5.04 ± 0.96	6.09 ± 0.87	6.21 ± 0.93	6.16 ± 0.85

[a] Log_{10} ($cfu_{CASF} + cfu_{APT}$) g^{-1}.
[b] Log_{10} cfu g^{-1} counts (average ± SD) of heat-treated meat-products when the two unfit kavourmas samples are removed from the analysis.

The data from the cheese samples analysed in this study indicate higher total bacterial counts for the unripe, high-pH whey cheeses and lower counts for the hard-cheese category, with semi-hard and soft cheeses yielding intermediate values. In cheeses, the $\log_{10}$ cfu g^{-1} difference between average counts on PCA and on CASF was in the range of 1.5–1.9 indicating that despite the plethora of LAB in ripened products approximately one in every 30 ($10^{1.5}$)–80 ($10^{1.9}$) microorganisms present in these products respectively was a contaminant. Whey cheeses receive adequate heat-treatment during the whey-protein denaturation step and are in excellent microbiological status immediately following manufacture (Kandarakis, 1986). On the other hand, their high pH and high moisture content render these products suitable substrates for rapid growth of contaminating organisms, which in this case can be LAB originating from the dairy facility environment, as well as other pathogenic or non-pathogenic organisms (Kandarakis, 1986; Papageorgiou et al., 1996).

The data from fermented meat-products and sandwiches were analogous to those from cheeses with the exception that the contaminating microflora was found to constitute a higher proportion of the food microflora (approx. 1 in every 6 and 15 cells, respectively). The heat-treated meat-products category was the only one in which the contaminating microflora was the predominant bacterial population. This finding is not surprising because the primary cause of bacterial presence in this food category is post-pasteurization contamination.

Two items exceeded the allowable APC level of 5×10^4 cfu g^{-1} (this limit applies only to heat-treated meat-products (HPD 9/1989, 1989)) and were therefore deemed unfit for consumption. Both items (originating from different manufacturers and sampling dates) were samples of a traditional regional product (kavourmas) made of a mixture of comminuted meat (combinations of pork, beef or lamb) blended with spices. The product undergoes variable heat-treatment and is consumed after additional cooking by the consumer, or without any further treatment. The actual $\log_{10}$ cfu g^{-1} PCA values for the two products were 7.59 and 7.64 and the respective CASF values were 7.48 and 7.38. These products therefore had either received inadequate heat-treatment, or had been heavily contaminated after heating. These two items were distinctly different in bacterial load than the rest of the heat-treated meat-products examined in this study; both the average bacterial count and the respective S.D. of the heat-treated meat-product category are substantially reduced upon exclusion of these two items from the analysis (Table 1).

3.2. Food-borne pathogens in RTE foods

None of the 87 foods tested for *Salmonella* spp. and *L. monocytogenes* yielded positive results (the pathogens were not detected in 25 g). In addition, all 26 meat-products that were analysed for the presence of sulfite-reducing clostridia were within the tolerance levels (less than 10 g^{-1}). These findings are in agreement with the overall incidence (less than 1%) of these microorganisms in these types of RTE products as determined by examinations conducted over the years in the FWTL (unpublished data). Three out of the 87 foods tested (3.4%) exceeded the tolerance levels in one or more categories. The first two items belonged to the whey-cheese category: a sample of Myzithra, which yielded levels of coliform bacteria and *E. coli* of 6.48 and 2.90 $\log_{10}$ cfu g^{-1}, respectively and a sample of Anthotyros with coliform bacteria and *E. coli* counts of 5.48 and 5.30 $\log_{10}$ cfu g^{-1}, respectively. The $\log_{10}$ cfu g^{-1} counts of contaminating flora and total mesophilic flora for these two items were 7.07 and 7.99 (Myzithra), and 7.38 and 8.44 (Anthotyros), respectively. Therefore, in these items, approximately 1 in every 10 organisms present was a non-LAB contaminant. The third item was a sample of hard cheese (Kefalograviera), which was found to be heavily contaminated with *E. coli* (5.04 $\log_{10}$ cfu g^{-1}) and coagulase-positive *S. aureus* (3.60 $\log_{10}$ cfu g^{-1}). The $\log_{10}$ cfu g^{-1} counts of contaminating and total mesophilic flora for this sample were 6.39 and 6.54, respectively, indicating that the product was of inferior quality. All other cheese samples were found to be in accordance with the specified microbiological limits (CEC, 1992; HPD 56/1995, 1995).

3.3. Non-lactic acid, contaminating microbial flora in RTE foods

It should be emphasized that the intent of the present study was not to be strictly quantitative in nature. Nonetheless, the magnitude of the contaminating microflora levels in several of the food categories examined is a reason for concern. Growth on the CASF medium represents the non-lactic acid fraction of the food microflora. This microflora can be characterized as contaminating, because its identity is unknown and its presence in RTE foods is unintentional. Depending on the type of food, the origin of this microflora can be both endogenous and/or exogenous: in fermented meat-products and ripened cheeses the internal source is the microorganisms that are present in raw materials such as meat or milk (which in the case of cheese products survive the milk pasteurization process) and which are propagated to various extents during the stages of fermentation or ripening and subsequent cold storage. Non-pathogenic bacteria surviving the pasteurization process should be the main source of endogenous contaminating flora in heat-treated products. Finally, in both pasteurized and fermented RTE foods the possibility of (post-pasteurization) contamination (even with pathogenic bacteria) cannot be excluded. During

processing, such contamination could occur from utensils, packing materials, the factory environment and personnel due to inadequate hygiene.

The magnitude of the contaminating flora in RTE foods at the point of sale depends on the microbiological quality of the starting materials, the overall conditions of hygiene during manufacture and the conditions of cold storage. It may be advisable therefore, to take into consideration the contaminating microflora counts, upon evaluating the quality of RTE foods. The existing legislation in Greece regarding RTE foods is not aimed towards this goal. The only established provision (besides the ones in effect for pathogenic bacteria and indicator organisms) as mentioned above is that heat-treated meat-products should not contain an APC in excess of 5×10^4 cfu g^{-1} ($\log_{10}$ cfu $g^{-1} = 4.7$) (HPD 9/1989, 1989). This requirement does not apply for fermented (meat or dairy) products probably based on the rationale that an APC result would be a meaningless representation of the natural LAB flora used in these products. In this communication, we have shown that the LAB and contaminating microflora populations in RTE foods can be differentiated, because the contaminating microflora can be selectively enumerated using a peptone-based sugar-free medium (IDF, 1991); such media are available by more than one company under slightly different names. Therefore, enumeration of the contaminating micro-flora would be a sensible solution for evaluating (micro-biological) quality in RTE fermented products, analogous to the APC criterion used for heat-treated products. Potential guidelines, that could be set by regulatory agencies or adopted by individual manufacturers, should be flexible in that they should reflect the different stages of the food in the production and distribution chain (purchasing of raw materials, production of the food under good manufacturing conditions, expected shelf-life in the retail market). An analogous approach has been previously proposed for pathogen testing (Institute of Food Science and Technology (IFST), 1997). This scheme would provide the food industry and regulatory agencies with an overall index of sanitation in the food production chain and a tool for monitoring subsequent temperature abuse in the distribution chain and retail market. In fact, such an approach would be beneficial for the consumer too. Based on screening for pathogens and indicator organisms, several items examined as part of this study were found appropriate for consumption, when in fact, the magnitude of their contaminating flora, which exceeded 10^6 or in some cases 10^7 bacteria g^{-1}, indicated that they were either manufactured using poor-quality starting materials, processed under unhygienic conditions, or temperature-abused during transportation and storage.

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