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Elaboration of Microbiological Guidelines as an Element of Codes of Hygienic Practices for Small and/or Less Developed Businesses To Verify Compliance with Hazard Analysis Critical Control Point

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ABSTRACT

Effective assurance of microbiological food safety practices in small and/or less developed businesses is not yet resolved. Although a start has been made by drafting hygiene codes, feasible methods for verifying manufacturing processes that rely on strict and meaningful criteria to be applied to process points are still lacking. This investigation is a model study with various types of ready-to-eat foods aimed at verifying adequate processing for safety and subsequent meticulous hygienic handling and safe storage of foods in small and/or less developed businesses by the use of quantitative methods for selected indicator organisms, as standardized by the International Organization for Standardization. The manufacture of the foods that were used in this study invariably included (i) a heat processing step that ensured a level of lethality of nonsporing organisms at least equivalent to the reduction of such organisms attained in the pasteurization of milk and (ii) effective means of prevention of postprocess recontamination and recolorization. The results of this study indicate that simple microbiological criteria used for this purpose, including aerobic mesophilic colony (standard plate) counts, *Enterobacteriaceae* counts, and, in some instances, enumeration of yeast propagules, allow adequate verification of good practices throughout. This verification through monitoring of samples taken during processing in small and/or less developed businesses was found to be an attractive alternative to the conventional examination of end products.

It has been well established scientifically and generally accepted by the field that microbiological food integrity assurance *(30)* (i.e., safety and nutritional and sensory quality) by retrospective monitoring of marketed samples is an unavailing effort. Instead, a forward control approach, i.e., a proactive hazard analysis establishing critical control points policy, has to be adopted *(20)*. However, hazard analysis critical control point (HACCP) sensu stricto is by no means a panacea, unless longitudinally integrated (i.e., starting at the farm or from marine sources and carried through to the moment of consumption) *(28)*. This maxim has been implemented by drafting and adopting formally approved codes of practices (ACoPs), specifically developed for each industry sector by the Food and Agriculture Organization/World Health Organization Alimentarius Committee *(23)*. Within the European Union (EU), this approach has been adopted by the European Commission *(15)*, and adherence to the HACCP system is required by EU Hygiene Directive 93/43 *(4)*. The currently proposed draft of the EU regulation ''Hygiene of Foodstuffs'' *(10)* contains guides to good practice for hygiene and application of HACCP principles, which appropriately describe

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TABLE 1. *Selected food preparation processes*

Preparing soups and sauces
Preparing cooked rice, rice, and pasta dishes (Chinese food)
Preparing stew/hotchpot (cooked and mashed potatoes with cooked vegetables)
Cooking various meats (pork, beef, lamb, chicken, medium-heated meat, e.g., roast beef)
Frying fish
Boiling, unshelling, and slicing of eggs
Preparing desserts (custards)
Preparing pastry
Preparing Russian salads

TABLE 2. *Sampling points*

A	Immediately after heating
B	After cooling to 6 ± 1°C
C	After keeping at 67 ± 2°C, as in catering and hot vending
D1	After 24 h of refrigerated storage (6 ± 1°C)
D2	After 48 h of refrigerated storage (6 ± 1°C)
D3	After 72 h of refrigerated storage (4 ± 1°C)
E	After reheating to 75 ± 2°C, as when preparing meals in advance

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TABLE 3. *Methods used*[a]

Target (CFU g^{-1})	Reference	ISO no.	Medium	Incubation	
				Temperature (°C)	Time (h)
Aerobic mesophilic colonization	*9*	4833	PCA	30	72
Enterobacteriaceae					
Meat and meat products	*6*	5552	VRBG	37	18
All other products	*3*	7402	VRBG	37	18
Yeasts					
Meat and meat products	*5*	13681	OGGY	25	120
All other products	*2*	7954	OGY	25	120

[a] ISO, International Organization for Standardization; PCA, plate count agar; VRBG, violet red bile glucose agar; OGGY, oxytetracycline gentamicin glucose yeast extract agar; OGY, oxytetracycline glucose yeast extract agar.

codes of hygienic practice that encompass HACCP application.

An important but currently unresolved problem is the effective application of such hygiene codes in small and/or less developed businesses (SLDBs), especially in restaurants and local shops. The need for this policy has been addressed by the present authors *(29)* and subsequently recommended by an expert consultation convened by the World Health Organization *(7)*. The solution relies on the elaboration of crystal-clear ACoPs written in simple language and accompanied by an appropriate formal structural training.

It remained to be investigated how to assess success or failure in the implementation of ACoPs in SLDBs. In the large-scale food production and catering establishments, verification of adherence to HACCP, including audits and microbiological spot testing *(34)*, is often performed according to the HACCP Pilot Program of the Food and Drug Administration *(24)*. On the other hand, in smaller operations HACCP awareness is still low and monitoring for success mostly lacking *(17)*. The aim of this investigation is to assess the possibility of elaborating and adopting simple verification procedures for application by SLDBs, including the introduction of microbiological criteria. Numerical limits of the latter, indicating safety of production processes are outlined in food safety objectives *(18, 22, 32)*.

MATERIALS AND METHODS

Study design. Although food preparation practices in smaller businesses show considerable variation, on a closer look, many similarities can yet be observed in frequently used preparation processes. Based on that observation, a number of common pro-

TABLE 4. *Sample point A: sampling immediately after heating*[a]

Processed foods	*n*	Mesophilic colony count (CFU g^{-1})			*Enterobacteriaceae* (CFU g^{-1})		Yeast propagules (CFU g^{-1})	
		10^3	10^4	10^5	10	10^2	10^2	10^3
Broth	17	+	+	+	+	+	+	+
Soups	17	+	+	+	94	+		
Sauces	48	94	+	+	+	+		
Rice	17	+	+	+	+	+		
Rice dishes	19	63	89	+		+	+	+
Potatoes for hotchpot	19	+	+	+	+	+	+	+
Meat								
Pork	20	85	*	+	90	+		
Beef	20	85	*	+	+	+		
Lamb	20	90	90	+	+	+		
Chicken	20	90	+	+	+	+		
Moderately heated	20	75	90	+	+	+		
Fish								
Fried	21	+	+	+		+	+	+
Salted and fried pouch of cod	20	+	+	+		+	+	+
Spiced after frying	20	68	+	+		+	+	+
Desserts	15	93	+	+	+	+	+	+
Mashed potatoes for cold salads	15	+	+	+				

[a] Percentage of samples complying with criteria; +, 100%; *, ≥95%.

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TABLE 5. *Sample point B: sampling immediately after cooling to 6°C (±1°C)*[a]

Processed foods	*n*	Mesophilic colony count (CFU g^{-1})				*Enterobacteriaceae* (CFU g^{-1})		Yeast propagules (CFU g^{-1})		
		$<10^3$	$<10^4$	$<10^5$	$<10^6$	$<10^2$	$<10^3$	$<10^2$	$<10^3$	$<10^4$
Soups	17	+	+	+	+	+	+	+	+	+
Sauces	48	86	+	+	+	+	+	+	+	+
Rice	17	53	67	+	+	+	+	+	+	+
Rice dishes	19	47	63	89	+	+	+	+	+	+
Hotchpot										
Potatoes	19									
Mashed potatoes with spices added	20	74	*	+	+	+	+	+	+	+
After adding cooked vegetables	19	53	84	+	+	+	+	+	+	+
Meat										
Pork	20	*	+	+	+	+	+			
Beef	20	75	+	+	+	+	+			
Lamb	20	85	+	+	+	+	+			
Chicken	20	85	+	+	+	+	+			
Moderately heated meat	20	75	+	+	+	+	+			
Heated minced meat	20	76	94	+	+	94	+	+	+	+
Fish										
Fried	21	90	+	+	+	+	+	+	+	+
Salted and fried pouch of cod	20	*	+	+	+	+	+	+	+	+
Spiced after being fried	20	75	+	+	+	+	+	+	+	+
Eggs (after unshelling)	16	88	+	+	+	+	+	+	+	+
Desserts	15	87	+	+	+			+	+	+
Pastry										
With whipped cream	25	56	92	+	+	85	*	85	*	+
With cream	20	60	90	90	+	90	+	84	+	+
Salads										
Mashed potatoes	15	60	80	+	+	93	+	88	88	+
After adding cooked vegetables	16	20	67	94	+	81	94[b]	87	+	+
After adding raw vegetables	16	13	56	89	+	88	94[b]	88	94	+

[a] Percentage of samples complying with criteria; +, 100%; *, ≥95%.
[b] 100% of the samples contained less than 10^4 CFU/g.

cesses were selected to assess colonization levels in (semimanufactured) products sampled at several sequential stages. For the assessment of each process, at least 15 and preferably 20 different operations were selected. Immediately before samples were drawn, an experienced food inspector audited the operations concerned. The condition for including an operation in the investigation was its strict compliance with the specific ACoP.

Foods studied. The broad variety of foods examined in this investigation included mostly ready-to-eat (RTE) commodities.

TABLE 6. *Sample point C: sampling after keeping at ≥65°C for 2 h (ready to eat)*[a]

Processed foods	*n*	Mesophilic colony count (CFU g^{-1})				*Enterobacteriaceae* (CFU g^{-1})	
		$<10^2$	$<10^3$	$<10^4$	$<10^5$	$<10^2$	$<10^3$
Soups	17	+	+	+	+	+	+
Sauces	48	+	+	+	+	+	+
Rice	17	94	+	+	+	+	+
Hotchpot (ready to eat)	19	74	89	+	+	+	+
Fish							
Fried	21	+	+	+	+	+	+
Salted and fried pouch of cod	20	+	+	+	+	+	+
Spiced after being fried	20	20	90	+	+	+	+

[a] Percentage of samples complying with criteria; +, 100%.

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TABLE 7. *Sample point D1: sampling after 24-h storage at 6°C (±1°C)*[a]

Processed foods	*n*	Mesophilic colony count (CFU g^{-1})				*Enterobacteriaceae* (CFU g^{-1})		Yeast propagules (CFU g^{-1})		
		$<10^3$	$<10^4$	$<10^5$	$<10^6$	$<10^2$	$<10^3$	$<10^2$	$<10^3$	$<10^4$
Heated potatoes for hotchpot	19	94	+	+	+	+	+	+	+	+
Meat (heated minced meat)	17	82	94	+	+	+	+	+	+	+
Fish										
Fried	21	90	+	+	+	+	+	+	+	+
Salted and fried pouch of cod	20	+	+	+	+	+	+	+	+	+
Spiced after being fried	20	+	+	+	+	+	+	+	+	+
Pastry										
With whipped cream	25	77	+	+	+	+	+	61	94	+
With cream	20	63	89	+	+	+	+	89	+	+

[a] Percentage of samples complying with criteria; +, 100%.

The various preparation processes are listed in Table 1. All processes included a processing-for-safety step. This step consisted of (i) heating to ensure at the food's coldest spot a level of lethality of nonsporing bacteria and eukaryotes at least equivalent to that attained in milk pasteurization (i.e., 2 min at 72°C) *(29)*; (ii) strict precautions to avoid postprocess recontamination by adherence to measures of meticulous operational hygiene; and (iii) the control of recolonization by rapid cooling to and subsequent storage and distribution at food temperatures in the ranges of either 4 ± 1°C or 6 ± 1°C.

The heat dissipation incurred in the applied mode of processing for safety of RTE foods ensured adequate devitalization of any level of initial contamination of nonsporing microorganisms in raw materials of good commercial quality. As far as health risks are concerned, this is substantiated by literature data on thermal resistance of pathogenic microorganisms of significance in foods *(21)*. As addressed below, the adequacy of the pasteurization step applied to foods in this study is further supported by the most reassuring evidence accrued in the dairy industry. When raw milk was treated strictly in accordance with this processing scheme, it

TABLE 8. *Sample point D2: sampling after 48 h storage at 6°C (±1°C)*[a]

Processed foods	*n*	Mesophilic colony count (CFU g^{-1})				*Enterobacteriaceae* (CFU g^{-1})		Yeast propagules (CFU g^{-1})		
		$<10^3$	$<10^4$	$<10^5$	$<10^6$	$<10^2$	$<10^3$	$<10^2$	$<10^3$	$<10^4$
Soups	17	82	94	+	+	+	+	+	+	+
Sauces	48	88	*	+	+	90	+	+	+	+
Rice	17	53	65	+	+	+	+	+	+	+
Rice dishes	19	47	68	79	+	+	+	+	+	+
Hotchpot										
Potatoes	19	89	89	+	+	+	+	94	+	+
Mashed potatoes with spices added	20	75	*	+	+	+	+	+	+	+
After adding cooked vegetables	19	89	+	+	+	+	+	+	+	+
Meat										
Pork	20	85	90	+	+	90	+	+	+	+
Beef	20	90	+	+	+	+	+	+	+	+
Lamb	20	65	90	*	+	*	+	+	+	+
Chicken	20	85	+	+	+	+	+	+	+	+
Moderately heated meat	20	84	84	+	+	+	+	+	+	+
Heated minced meat	17	76	94	94	+	+	+	94	+	+
Eggs										
After unshelling	16	94	+	+	+	+	+	+	+	+
After slicing	16	+	+	+	+	94	+	+	+	+
Desserts	16	88	+	+	+	+	+	+	+	+
Salads										
After adding cooked vegetables	16	21	50	93	+	86	93[b]	43	71	93[c]
After adding raw vegetables	16	14	50	93	+	86	93[b]	50	79	+

[a] Percentage of samples complying with criteria; +, 100%; *, ≥95%.
[b] 100% of the samples contained less than 10^4 CFU g^{-1} of *Enterobacteriaceae.*
[c] A total of 100% of the samples contained less than 10^5 CFU g^{-1} of yeast propagules.

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TABLE 9. *Sample point D3: sampling after 72-h storage at 4°C (±1°C)*[a]

Processed foods	*n*	Mesophilic colony count (CFU g^{-1})				*Enterobacteriaceae* (CFU g^{-1})		Yeast propagules (CFU g^{-1})		
		$<10^3$	$<10^4$	$<10^5$	$<10^6$	$<10^2$	$<10^3$	$<10^2$	$<10^3$	$<10^4$
Broth	17	94	+	+	+	+	+	+	+	+
Rice	17	47	59	82	+	94	+	+	+	+
Meat										
Pork	20	89	+	+	+	90	+	+	+	+
Beef	20	70	+	+	+	+	+	+	+	+
Lamb	20	70	90	+	+	+	+	90	90	+
Chicken	20	25	60	+	+	+	+	+	+	+
Moderately heated meat	20	60	80	+	+	+	+	+	+	+
Eggs (sliced)	16	13	+	+	+	+	+	+	+	+
Desserts	16	75	88	+	+	+	+	+	+	+

[a] Percentage of samples complying with criteria; +, 100%.

was never involved in infectious enteritis, previously frequently observed on ingestion of raw milk and dairy products, such as soft curd cheeses manufactured from raw milk *(27)*.

Selection and determination of target organisms. The extent of management of colonization of RTE foods can be assessed from enumeration of appropriate marker organisms *(11, 12, 19, 25, 26)*. These are divided into indicator and index organisms. When numbers of indicator organisms (CFU) remain acceptable, it can be inferred that the production process or processes are satisfactory. Acceptable numbers are those attainable by meticulous compliance with codes of practice and derived from the identification, characterization, and management of microbial hazards encompassed by HACCP. The other group of marker organisms, index organisms *(19)*, shares significant ecological and physiological characteristics with target, foodborne pathogenic organisms. Their detection in unacceptable numbers therefore provides evidence of potential presence of pathogens.

Acceptable numbers of indicator organisms in RTE foods result from surveys on samples, originating from operations, that comply with the HACCP strategy; hence, assessing accordance with these levels provides assurance that any pathogens potentially present in the raw materials have been reduced to tolerable limits in the product as marketed. The food is consequently safe to eat even when nonsporing pathogens with very low infectious ranges might have contaminated one or more of the raw materials. Eight decades of experience in the dairy industry confirms that this assumption is justified. By ensuring that numbers of well-established indicator organisms (coli aerogenes bacteria) remained acceptable, this industry ensured that their products consistently ceased to cause foodborne disease.

Process verification based on the monitoring of appropriate indicator organisms in RTE foods may also be valuable as a substitute for monitoring of pathogens. Relying on negative results of testing for pathogens constitutes an inappropriate strategy when verifying microbiological safety of processed foods *(1)*. Because pathogens are occurring in very low numbers, compounded by not being distributed homogeneously throughout processed foods, failure to detect them does not indicate their absence. On the other hand, finding acceptable numbers of indicator organisms in similar sampling regimens is much more reliable. Low values for performance criteria indicate process control and, consequently, a high probability of tolerable low incidence of pathogens *(30)*. However, championing the use of indicator organisms does not detract from the necessity, in some occasions, to seek pathogens or appropriate index organisms in some RTE foods. Such instances include outbreak investigations *(35)* and specific requests from customers in the framework of the vendor assurance policy *(28)*.

Methods of examination. Samples were drawn at the production sites A through E as in Table 2. These sites were selected because they constitute the critical points and areas within longitudinally integrated operations *(30)*. The food specimens were stored for no longer than 18 h at 0 to 4°C before being examined. In the present investigation, selected indicator organisms included aerobic mesophilic colony (standard plate) count and enumeration of *Enterobacteriaceae* (CFU g^{-1}). When the ecological determinants of particular foods would allow yeasts to obtain a rapid footing *(31)*, an enumeration of numbers of yeast propagules was conducted. As is customary in European Public Health Food Inspection Laboratory Services, standard operating procedures, elaborated under the auspices of the International Organization for Standardization (ISO), were used throughout (Table 3). It was verified that, for this purpose, ISO standard operating procedures would provide results entirely compatible with those included for these criteria in the American Public Health Association compendium *(16)*.

All colony counts were grouped at levels of 10 to 10^6 CFU g^{-1}, depending on the prevailing British guidelines *(13)*. These guidelines generally rely on the order of 10^4 g^{-1} for total aerobic colonization and 10^2 g^{-1} for *Enterobacteriaceae* and, where appropriate, for yeast propagules.

RESULTS

In Tables 4 through 10, the data are recorded based on compliance with the chosen categorization of colonization levels. These figures demonstrate that freshly cooked foods are virtually always colonized at a level below 10^4 CFU g^{-1}, whereas *Enterobacteriaceae* levels invariably are below 10^2 CFU g^{-1} and as a rule even below 10 CFU g^{-1}. Yeasts were never encountered at levels exceeding 10^2 CFU g^{-1}. Heating to achieve the recommended count reductions *(29)* can therefore be considered as having been adequately performed, and the adopted criteria seem hence to be warranted for the verification at this stage of food preparation.

Cooling of heated foods of particular commodities

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TABLE 10. *Sample point E: sampling after reheating the foods at ≥75°C after being held at 6°C (±1°C) for 24 or 48 h*[a]

Processed foods	*n*	Mesophilic colony count (CFU g^{-1})				*Enterobacteriaceae* (CFU g^{-1})		Yeast propagules (CFU g^{-1})
		$<10^2$	$<10^3$	$<10^4$	$<10^5$	$<10^2$	$<10^3$	$<10^2$
Soups	15	+	+	+	+	+	+	+
Meat (heated minced meat)	34	*	+	+	+	+	+	+

[a] Percentage of samples complying with criteria; +, 100%; *, ≥95%.

caused slight increases in the numbers of microorganisms. However, in virtually all instances, total colony counts remained below 10^5 CFU g^{-1} and *Enterobacteriaceae* counts below 10^2 CFU g^{-1}. Not surprisingly, only when ingredients such as raw or cooked vegetables and spices were added to foods after heating, an increase to the extent of one log cycle was noticed, originating from spores in case of cooked vegetables and specific association of raw foods and from handling the foods during preparation.

Refrigerated storage in compliance with the ACoP produced a substantial increase in colony counts, especially when handling of meats and the addition of vegetables were taken into account. Some increase was already notable after cooling (Table 5). Hot holding did not markedly affect colonization; reheating to 75°C yielded the results as favorable as could be expected.

DISCUSSION

Controlling temperature, pH, and water activity of foodstuffs attempts to keep numbers of viable microorganisms at an acceptable level, especially those microorganisms that deteriorate foods or pose a threat to human health *(30)*. Hence, monitoring these food attributes constitutes the first priority in verifying the efficacy of any adopted measures of food protection. Microbiological testing may play an important role in process verification, although its limitations must be taken into account *(8, 13, 14, 16, 33)*. As addressed previously *(1)*, it is an illusion to assume that a search for pathogenic microorganisms in processing foods constitutes an effective procedure for the verification of the microbiological safety. Rather, enumeration of suitable marker organisms has been adopted in the dairy industry *(27)* and should be incorporated when verifying control in SLDBs *(29)*.

This investigation of more than 1,600 samples demonstrates that when ACoP-defined procedures are faithfully followed, food of good microbiological quality can be consistently produced. Ecologically and strategically relevant criteria for verification of process control have been recently suggested for general use. This investigation demonstrates that these criteria are also attainable and maintainable in SLDBs when set at mesophilic colony counts not substantially exceeding 10^4 CFU g^{-1} and *Enterobacteriaceae* and, where ecologically pertinent, yeast propagule counts not markedly exceeding 10^3 CFU g^{-1}. Since, as addressed previously, these limits ensure adequate consumer protection, our data demonstrate that foods, when adequately heated and subsequently protected from recontamination and recolonization in compliance with the ACoPs, will maintain their paucimicrobial condition required for consumer protection. Exceeding the suggested limits signals insufficient process control and thus a potentially elevated risk for the consumer, which calls for immediate rectification of existing hiatuses and breaches in safe practices.

The results of this survey also constitute a most welcome support in auditing food and catering premises in the context of enforcing the regulations, calling for checking critical sites of the manufacture for conformity with ACoPs.

These criteria should obviously be handled with caution and are to be exercised by a microbiologically trained staff. For instance, savory meals may contain raw vegetables, whereas fresh cream is often added to pastry. In those instances where ingredients not fully processed for safety are incorporated, *Enterobacteriaceae* as process markers have to be replaced by *Escherichia coli* as the valid indicator of fecal contamination *(26)*. Also, for statistical reasons, a single sample that shows slightly elevated counts should not be considered to indicate overall lack of control until data on further samples endorse this conclusion. Further investigations are in progress. These studies include an evaluation of the need for additional microbiological criteria *(13)* to be applied to the commodities studied with the purpose of meeting food safety objectives, applying by definition to their condition on the moment of ingestion. In addition, the suitability of our database for incorporation in Plumb's maxim of vendor assurance *(28)* will be exploited to further improve manufacturing processes in SLDBs and thus provide consumers with foods of the best attainable safety and quality.

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