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An identification procedure for foodborne microbial hazards

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Abstract

A stepwise and interactive identification procedure for foodborne microbial hazards has been developed in which use is made of several levels of detail ranging from rough hazard identification to comprehensive hazard identification. This approach allows one to tackle the most obvious hazards first, before focusing on less obvious hazards. The interactive character of the identification procedure is based on the use of several knowledge sources. Combination of knowledge sources, expressed in the use of knowledge rules, supports the user in systematically selecting hazards which may pose a real risk to the consumer. Due to the structured method and the clear definitions of the knowledge rules, the procedure is transparent and may be changed if necessary. The hazard identification procedure has been implemented as a computer program, resulting in a decision-supporting identification system. It provides a way to efficiently assess those hazards which may cause harm if not brought under control during processing. The procedure forms a basis for quantitative risk assessments. © 1997 Elsevier Science B.V.

Keywords: Microbial hazard identification; Pathogen; HACCP; Quantitative risk assessment

1. Introduction

The HACCP (Hazard Analysis Critical Control Points) system was developed in the early 1970s. The system is used to manage the safety of food products systematically by paying special attention to those steps in the process that are essential in the production of acceptably safe foods. In the recent past, many food processing companies have introduced safety management systems based on HACCP principles. Application of the principles of HACCP has become mandatory for food companies in the European Community (EC, 1993). The HACCP system is however often used qualitatively and subjectively. A quantitative approach of the HACCP system provides a better way to set proper criteria for critical process steps (indicated as CCPs), to execute control measures, and to optimise processes according to a certain risk. The quantitative approach can be created by the implementation of quantitative risk analysis (QRA) in existing HACCP systems (Corlett and Stier, 1991; Notermans et al., 1994b; Buchanan, 1995).

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QRA is based on quantitative data and models and consists of six activities: 1) hazard identification, 2) exposure assessment, 3) dose-response assessment, 4) risk characterisation, 5) risk management, and 6) risk communication. Steps 1 to 4 are often termed risk assessment.

As shown in Table 1, hazard identification is the first activity in both QRA and HACCP. The importance of identification of hazards is mentioned in almost every reference dealing with QRA and HACCP. However, a systematic approach to the identification of hazards for food products is hardly described anywhere. Such an approach is deemed necessary to prevent pathogens relevant to products being disregarded and is especially necessary for newly developed and modified products, because new hazards may arise in these products. Only Notermans et al. (1994a) presented a general approach to the systematic identification of microbiological hazards for food products. This approach inspired the current development of a computer aided system for hazard identification. Our hazard identification procedure differs from Notermans' approach mainly by a stepwise identification of important hazards and its interactive character. Stepwise identification of relevant hazards is based on the use of three levels of detail ranging from rough hazard identification to comprehensive hazard identification. The interactive character results from systematically using several knowledge sources in identifying hazards. The knowledge sources are: literature knowledge, expert knowledge, and the user's knowledge.

1.1. QRA: terms and definitions

Several definitions for terms in QRA can be found in literature. For the purposes of this research, working definitions for *hazard* and *hazard identification* have been set up.

Hazard, in food production, is often defined as a substance that has the potential to cause harm (HACCP Working Group, 1992; CODEX, 1995). Hazard is also defined as an event, like unacceptable growth or survival of pathogens (ICMSF, 1988). In HACCP practice a combination of both definitions is often used. In describing the hazard identification procedure the first definition is used, so a hazard is considered to be a harmful substance instead of an event.

Hazard identification can be defined as the qualitative indication of potentially adverse health effects associated with exposure to foodborne agents (Rose et al., 1995; Potter, 1996). Notermans and Teunis (1996), and Bernard and Scott (1995) on the contrary, define hazard identification as a qualitative indication of the hazards that may be associated with the consumption of a particular food product. It is this latter definition that is used in this article.

2. An outline of the hazard identification procedure

The hazard identification procedure is shown in Fig. 1. The starting point of the hazard identification

Table 1
Steps in quantitative risk analysis (QRA) and in the HACCP system

QRA		HACCP (CODEX, 1995)	
1	Risk assessment Hazard identification	1	Hazard analysis: hazard identification, assessment of likelihood of occurrence of hazards and identification of preventative measures for their control
2	Exposure assessment	2	Determine CCP's
3	Dose-response assessment	3	Establish critical limits
4	Risk characterisation	4	Establish a monitoring system
5	Risk management	5	Establish corrective actions
6	Risk communication	6	Establish verification procedures
		7	Establish documentation

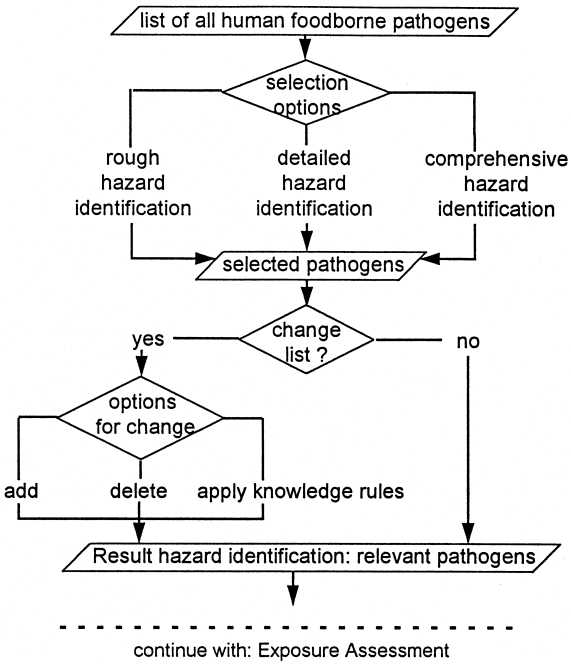

Fig. 1. Hazard identification procedure.

procedure is a list of microorganisms that are known to be pathogenic to man. Currently the list contains about 200 names of pathogens. Then three options can be selected: 1. rough hazard identification, 2. detailed hazard identification, and 3. comprehensive hazard identification. The process of consecutively using the levels of detail is illustrated in Fig. 2.

The reason for this approach is to perform risk assessments and control risks for the most relevant hazards before doing so for less expected hazards. The use of the levels of detail provides a way to maintain stepwise focus on the most important aspects with respect to risk assessment.

The detailed and comprehensive hazard identification may result in a long list of pathogens that is impractical to work with. It is efficient to start with the most relevant hazards of this list. The user can be supported in selecting these pathogens by the use of literature and expert knowledge. Literature knowledge is useful for selection of theoretically hazardous pathogens, whereas expert knowledge is useful to treat theoretical predictions with relativism. Literature and expert knowledge have been captured in knowledge rules. The user decides which knowledge rules are applied in the hazard identification. It is this combination of various knowledge sources that provides the dynamic and interactive character to the hazard identification procedure. The final result of the hazard identification procedure is a practical list of relevant pathogens. Risks can be assessed for these pathogens in the first instance.

In this article, the three levels of detail and the knowledge rules are described followed by the implementation of the hazard identification procedure as a decision-support system. Finally, the hazard identification procedure applied to several food products is described as an example.

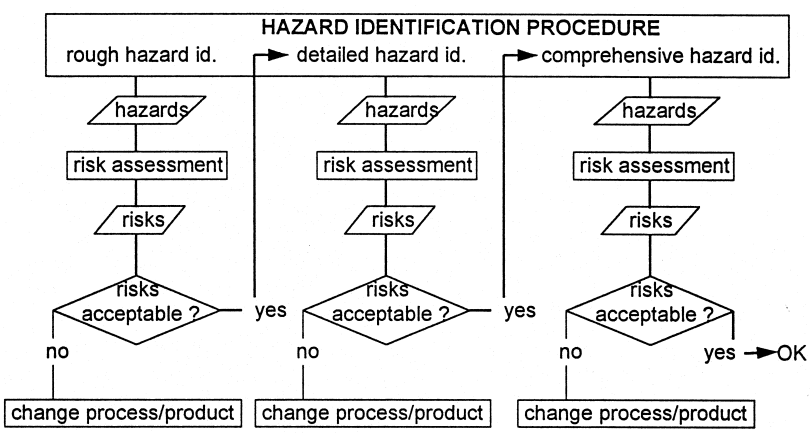

Fig. 2. Process of using several levels of detail in the hazard identification procedure.

3. Hazard identification at three levels of detail

3.1. Rough hazard identification

The rough hazard identification selects pathogens that were reported to have caused foodborne outbreaks in the selected food product in the past. These pathogens are the most obvious since they have caused health problems via the specified product, whereas other pathogens did not. Much data on foodborne-outbreaks and related pathogens can be found in literature (e.g. Bean and Griffin, 1990; Todd, 1992). Only a small proportion of all foodborne illness has however been reported to the authorities (CAST, 1994; Notermans et al., 1994a), and it has often been very difficult to determine which pathogen in which food item was the true causative agent at the moment of consumption. Moreover, food products often contain a variety of ingredients that could have been the source of the causative agents, yet foodborne outbreaks are mostly listed only under the food product (Bean and Griffin, 1990). However, if a case has been reported for a specified product it is reasonable to start a risk assessment for the causative pathogen.

3.2. Detailed hazard identification

The detailed hazard identification selects pathogens that have been reported as being present in the ingredients of the specified product. In literature many data can be found on ingredients with associated pathogens (e.g. Ayres et al., 1982; Jay, 1992). Pathogens that have been introduced into the product by ingredients may cause health problems if the production process is not properly controlled.

3.3. Comprehensive hazard identification

The comprehensive hazard identification procedure identifies all human pathogens as hazardous. By this means, pathogens that unexpectedly recontaminate the product can be included. The cases of previously unknown contamination of dried infant formula with *Enterobacter sakazakii* in 1989 (Bierling et al., 1989; Simmons et al., 1989) are examples of unexpected hazards. It was suspected that infant formula had been contaminated during the manufacturing process. The reservoir and mode of transmission of *Enterobacter sakazakii* has however not been clearly identified (Nazarowec-White and Farber, 1997).

By risk assessments for unexpected hazards and unexpected events (failure analysis) it is possible to estimate the food safety consequences of the occurrence of unexpected events. In this way it is possible to get an impression of possible problems in the future and to deal with them pro-actively.

4. Knowledge rules to be used in hazard identification

Knowledge rules can be used to reduce an impractically long list of pathogens in a systematic and well-founded manner, such that the hazards that are of most likely relevance for the specific product can be assessed.

Three types of knowledge rules are used in the procedure (Table 2); 1, rules concerning presence or absence, and survival or inactivation of pathogens; 2, general rules on pathogen characteristics; and 3, rules concerning growth opportunities and toxin production.

Type 1 rules select pathogens that are present or able to survive in the end product. Type 1 rules can for example remove vegetative bacteria for a pasteurised product. Still, type 1 rules do not provide an exclusive list of relevant pathogens. A pasteurised product may be subject to recontamination after inactivation, leading to presence of vegetative pathogens in the end product, and failures in the pasteurisation process may allow survival of vegetative pathogens. Rules of type 1 do not take into account these aspects which do occur in practice. Nevertheless, rules of type 1 provide a list of relevant pathogens under normal and hygienic circumstances.

Type 2 rules select pathogens that are likely to cause problems in the food product in practice. For example, a pathogen that is very rarely transmitted by food is not likely to cause health problems as a result of consuming a food product, and is therefore removed from the list.

Type 3 rules select pathogens that are able to grow or produce toxin in the product. Ability to grow is

Table 2
Knowledge rules in the hazard identification procedure

Description
Type 1: Rules concerning survival of pathogens
If pasteurisation occurs in the production process: remove all vegetative bacteria that contaminated the product before the inactivation
If sterilisation or radappertisation occurs in the production process: remove all pathogens that contaminated the product before the inactivation
If drying occurs: remove *Campylobacter spp*. and *Vibrio spp*. that contaminated the product before drying.
If the brine concentration in the product exceeds 5% (w/w): Remove *Pseudomonas spp*. (Mitscherlich and Marth, 1984).
If the brine concentration exceeds 10% (w/w): Remove all pathogens except for *Staphylococcus aureus* and *Listeria monocytogenes* (Mitscherlich and Marth, 1984; Shapton and Shapton, 1991).
Type 2: Rules concerning general pathogen characteristics
Remove exotic pathogens that are not by nature present in your region. For the Netherlands these are: *Coxiella burnetii, Francisella tularensis, Vibrio parahaemolyticus, Vibrio vulnificus* (Havelaar, 1992).
Remove pathogens of which exposure is negligible in your region because of effective risk management. For the Netherlands these are: *Brucella spp., Mycobacterium bovis, Salmonella typhi, Vibrio cholerae* (Havelaar, 1992; ICMSF, 1996).
Remove micro-organisms of which foodborne pathogenicity is uncertain: *Acetobacter spp., Acinetobacter calcoaceticus, Actinomyces spp., Aeromonas spp., Aeromonas caviae, Aeromonas hydrophila, Aeromonas sobria, Alcaligenes faecalis, Bacillus anthracis, Bacteroides melaninogenicus, Branhamella catarrhalis, Brucella spp., Brucella canis, Campylobacter fetus subsp. fetus, Chlamydia psittaci, Chlamydia trachomatis, Chromobacterium violaceum, Citrobacter spp., Citrobacter freundii, Clostridium bifermentans, Clostridium cadaveris, Clostridium carnis, Clostridium histolyticum, Clostridium limosum, Clostridium septicum, Clostridium sordellii, Corynebacterium diphteriae, Corynebacterium pseudotuberculosis, Coxiella burnetii, Dermatophilus congolensis, Edwardsiella tarda, Enterobacter spp., Enterobacter aerogenes, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium. Erysipelothrix rhusiopathiae, Flavobacterium meningosepticum, Franciscella tularensis, Haemophilus influenzae, Hafnia alvei, Helicobacter pylori, Klebsiella spp., Legionella pneumophila, Leptospira spp., Morganella morganii, Mycobacterium bovis, Nocardia farcinica, Plesiomonas shigelloides, Proteus spp., Proteus mirabilis. Proteus vulgaris, Providencia spp., Providencia alcalifaciens, Pseudomonas aeruginosa, Serratia liquefaciens, Serratia marcescens, Stachybotrys atra, Streptobacillus moniliformis, Streptococcus agalactiae, Streptococcus pyogenes, Yersinia pseudotuberculosis* (Riemann and Bryan, 1979; Koburger and May, 1982; Mitscherlich and Marth, 1984; Klein et al., 1991; Varnam and Evans, 1991; Todd, 1992; ICMSF, 1996).
Remove pathogens that rarely cause problems in man: *Brucella canis, Chromobacterium violaceum, Corynebacterium pseudotuberculosis, Coxiella burnetii, Dermatophilus congolensis, Listeria ivanovii, Listeria seeligeri, Listeria welshimeri, Pseudomonas cocovenenans, Streptococcus bovis, Streptococcus dysgalactiae, Streptococcus equisimilis* (Riemann and Bryan, 1979; Mitscherlich and Marth, 1984; ICMSF, 1996).
Type 3: Rules concerning growth opportunities of pathogens
Remove pathogens that, according to their growth characteristics (based on pH, temperature, and water activity), cannot grow or produce toxin in the end product, except for *Salmonella spp., Listeria spp., Shigella spp*. a.o.

based on the use of the minimum and maximum growth temperature, pH, and water activity. Other growth determining factors such as nitrite-content, bactericides etc. are not taken into account, which mostly results in worst-case estimations. Selection on growth possibilities is useful for the reason that exposure to pathogens in general is higher if pathogens did multiply in the consumed food product than if they did not, which generally results in higher probabilities of food infection and food poisoning. This is confirmed by various dose-response relations of pathogens (Teunis et al., 1996). Not all pathogens have known growth characteristics however, which presents problems for selection on the basis of growth opportunities. It is a fact that the most important pathogens, such as *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium perfringens*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella spp*., *Staphylococcus aureus*, and others, do have known growth characteristics. Also, unknown growth characteristics of pathogens may be replaced by known growth characteristics of

related pathogens. For example, the unknown growth characteristics of *Salmonella dublin* can be substituted by the rough growth characteristics of *Salmonella spp*. The non-availability of growth characteristics can therefore be handled, but should be done with caution. By using all types of rules, pathogens are selected that 1, are present and survive in the end product; 2, are likely to cause health problems in practice; and 3, are able to grow in the end product. It is important to perform risk assessments for the pathogens selected by these procedures.

If a strict first analysis to determine the most obvious hazards does not result in an answer, a less strict procedure is the next step. The user is free to choose which types of knowledge rules are used in the hazard identification, as there is no rank order of significance for the types of rules.

Some redundancy and inconsistency exists in the knowledge rules. According to the knowledge rule 'Remove microorganisms of which foodborne pathogenicity is uncertain', all species of a genus (for example *Klebsiella spp*.) have to be removed, as well as explicitly mentioned species (for example *Klebsiella pneumoniae*). In this example *Klebsiella pneumoniae* should actually not be mentioned in the knowledge rule. This problem of redundancy is explained in the description of the food database.

The knowledge rules are clearly defined in the hazard identification procedure, and as the definition is explicit, the rules may be criticised, and changed if necessary. Inconsistencies and new developments can therefore be handled easily.

To apply the knowledge rules properly, the hazard identification procedure must be used by experienced microbiologists. Only this will assure an efficient assessment of the most relevant hazards for a product, at each level of detail. The problem of hazard identification is too important and too complex to entrust to a stand alone system. The experienced microbiologist is supported in his decisions by the best use of literature and expert knowledge. Also, the use of literature and expert knowledge may provide the experienced microbiologist with new ideas or renewed insights into products and production processes.

5. Decision supporting identification system for microbial hazards

For practical use it is very convenient to implement the interactive procedure as a decision support system. The literature and expert knowledge used in the hazard identification are captured in three databases: a food database, a pathogen database, and a knowledge database. In the following sections, the databases are described, and subsequently the working of the computer program is explained.

5.1. Food database

The food database introduced by Zwietering et al. (1992) contains physical characteristics of products and ingredients, which were derived from literature. Next to physical characteristics, the food database (Database 1) is extended with information on presence of (groups of) microorganisms, and information on foodborne outbreaks in the past (Table 3), also derived from literature. All foods have an identification code (ID) that determines the position of the

Table 3
An example of the information stored in the food-database

Field	Sample data
Name	raw cow's milk
Code	S.A.A.A.A
pH	6.5
Temperature (T)	7
Water activity (a_w)	0.98
Oxygen availability	aerobic
Include groups of microorganisms	Coliforms
Include microorganism	*Actinomyces spp., Aeromonas hydrophila,* ... etc.
Outbreak related pathogen	*Campylobacter jejuni, Salmonella spp.* ... etc.

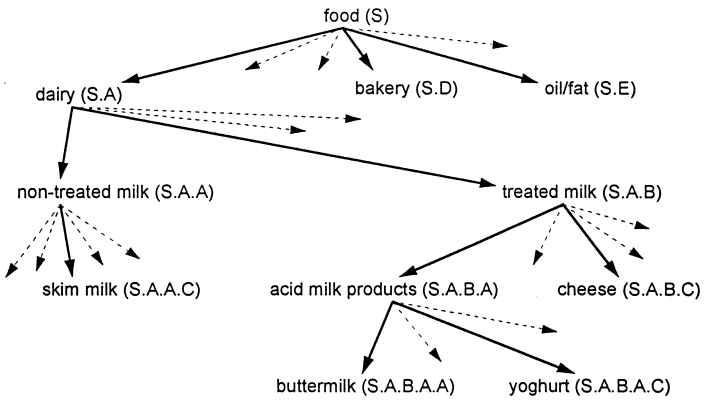

Fig. 3. Structure of food database, in which foods (with identification code) are classified (Zwietering et al., 1992).

food in the product classification tree (Fig. 3). The number of foods is more or less infinite and, as may be expected, not for every product/ingredient information on all the subjects is known. The product classification tree can be used to find a substitute for the missing information.

In the tree, products are sorted with respect to their physical properties, so foods that are grouped together are closely related and information on comparable foods can be used.

Some database records contain redundant information. They contain microorganism genera, including all species, as well as explicitly mentioned species of the genus. For example, for the product raw cow's milk (S.A.A.A.A) the food database contains *Bacillus spp*. (ICMSF, 1988) as well as *Bacillus cereus* (Robinson, 1981; CAST, 1994), and *Bacillus subtilis* (Robinson, 1981). Actually, the species should not be mentioned, since they belong to *Bacillus spp*. Species are however explicitly mentioned next to genera in the database as the data come from various references. It is not likely that ICMSF (1988), which reported *Bacillus spp*. to be present in raw cow's milk, has studied occurrence of all *Bacillus spp*. in raw cow's milk. Most probably, several species of *Bacillus* have been shown to be present in raw cow's milk, which was briefly indicated by *'Bacillus spp.'*. A study that reports the presence of specific species in a product in general gives more certainty of the actual presence of the species than a report of the presence of a genus.

Table 4
An example of the information stored in the pathogen database

Field	Sample data
Name	*Yersinia enterocolitica*
Code	Yers01
Type	bacterium
Spores	no
Infectious	yes
Toxinogenic	no
pH_{min}	4.6
pH_{opt}	7
pH_{max}	9
T_{min}	0
T_{opt}	32
T_{max}	44
$a_{w,min}$	0.97
$a_{w,max}$	1
Oxygen	fac. anaerobic
Food	yes
Exotic	no
Exposure negligible in the Netherlands	no
Pathogenicity uncertain	no
Rarely caused problems	no
No problems in Western countries	no

5.2. Pathogen database

For prediction of microbial spoilage, Zwietering et al. (1992) developed an organism database. This organism database has been modified into a database that only contains data on pathogens, as the hazard identification procedure only concerns pathogenic microorganisms (Database 2). Next to names of pathogens, with type and family specification, and pathogen characteristics, there is information on practical relevance of pathogens. An example of the information is shown in Table 4. Non-foodborne

pathogens and pathogens that have not been conclusively proven to be foodborne are included since these may cause problems related to food safety in future.

5.3. Knowledge database

The knowledge database (Database 3) contains knowledge rules. Knowledge rules were developed from the literature, then experts in the field of food microbiology were asked for their opinion on these rules and the rules were changed and reworded accordingly. The knowledge rules stored in Database 3 are shown in Table 2.

5.4. The computer program for hazard identification

The computer program starts with selection of a product and product characteristics, and with construction of a process flowsheet. After this, the user must choose a level of detail for which the hazard identification procedure will be performed. A list of pathogens is the result of this first selection procedure. The list can be modified according to the user's demands. There are several options of changing the list: add pathogens, remove pathogens, and apply knowledge rules. Addition and removal of pathogens are purely based on the user's expertise. Knowledge rules can be used if the user needs support in shortening the list. The user decides which types of knowledge rules he uses. The knowledge rules belonging to the chosen types appear one by one if appropriate. By acceptance of a knowledge rule, pathogens are deleted from the list. Before removal however, the computer program provides warnings for several knowledge rules. Amongst the benefits of these warnings is the opportunity to take typical recontamination routes into account. For example, if the knowledge rule 'if pasteurisation occurs: remove all vegetative bacteria and viruses' (Table 2) appears, it can be accepted or neglected. If it is accepted, *Salmonella spp*. is among the pathogens that are removed from the list. Before the pathogens are removed however, the computer program warns that *Salmonella spp*. may cause problems if the food is of animal origin, because of recontamination by workers' hands (de Wit and Kampelmacher, 1981). If the warning is accepted, the pathogen is not removed.

The outcomes are derived by matching data from the databases. The process of matching data was described by Zwietering et al. (1992). If, for example, selection on growth characteristics (type 3 knowledge rule) is performed, the physical properties of the product in Database 1 are matched to the growth characteristics of pathogens in Database 2.

The final result of the hazard identification procedure is a list of pathogens, that, according to the user and the information from the databases, are hazardous.

6. Results

The hazard identification procedure was applied to vacuum packed cooked potatoes, cooked ham, and sterilised milk.

6.1. Cooked potato

The results of the first two levels of detail applied to vacuum packed cooked potatoes are shown in Table 5. First a rough hazard identification was performed, by which pathogens were assessed that were reported to have caused health problems related to cooked potato in the past. The pathogen database found *Clostridium botulinum* type A to be reported to have caused problems in the past in vacuum packed cooked potatoes. It is prudent to first evaluate the risk of this pathogen in the process, since this organism is likely to be the most obvious hazard. If the risk is assessed for this hazard, and it is found to be acceptable, a more detailed hazard identification should be performed based on pathogens present in the ingredients of cooked potatoes. The ingredients used for the production of vacuum packed cooked potatoes are potatoes. Also, water is considered to be an ingredient since potatoes are washed with water during the production process. 33 pathogens were selected to be present in the ingredients potatoes and water (Table 5). Since this list is quite large it is useful to make a selection within this list and first start with the most likely pathogens to cause problems. For this selection knowledge rules can be used. Table 5 shows the results of application of the various types of knowledge rules. Application of

Table 5
Results of the identification procedure applied to vacuum packed cooked potatoes and results after application of the three types of knowledge rules

Rough hazard identification	Detailed hazard identification	Knowledge rules			
		Type 1	Type 2	Type 3	Type 1, 2 and 3
Clostridium botulinum	*Aeromonas spp.*			X	
type A	*Alcaligenes spp.*		X		
	Bacillus spp.	X	X		
	Bacillus anthracis	X		X	
	Bacillus cereus	X	X	X	X
	Chromobacterium spp.		X		
	Clostridium spp.	X	X		
	Clostridium botulinum type A	X	X		
	Clostridium botulinum type B	X	X		
	Clostridium botulinum type E	X	X	X	X
	Clostridium botulinum type F	X	X	X	X
	Clostridium perfringens	X	X		
	Corynebacterium spp.		X		
	Enterococcus spp.		X		
	Escherichia coli		X	X	
	Flavobacterium spp.		X		
	Fusarium spp.		X		
	Klebsiella spp.				
	Klebsiella pneumoniae				
	Listeria monocytogenes		X	X	
	Nocardia spp.		X		
	Pasteurella multocida		X		
	Plesiomonas shigelloides				
	Pseudomonas spp.		X		
	Pseudomonas aeruginosa			X	
	Pseudomonas pseudomallei				
	Salmonella spp.		X	X	
	Serratia spp.		X		
	Shigella spp.		X	X	
	Staphylococcus spp.		X	X	
	Streptococcus spp.		X		
	Vibrio cholerae				
	Yersinia enterocolitica		X	X	

type 1 rules resulted in a list of 9 pathogens, application of type 2 rules resulted in a list of 25 pathogens, and application of type 3 rules resulted in a list of 12 pathogens. For application of type 3 rules it was assumed that the pH of cooked potatoes is 6.2 ± 0.1, the water activity is 0.98 ± 0.01 (ICMSF, 1988), and the temperature is 6 ± 1°C, assuming that the potatoes are stored chilled. The ranges in pH, temperature (T), and water activity (a_w) are used to compensate for uncertainties in pH, T, and a_w, of the product and inaccuracies in determining the minimal pH, T, and a_w, at which growth can occur.

The pathogens left after application of all knowledge rules are *Bacillus cereus*, *Clostridium botulinum* type E, and *Clostridium botulinum* type F. The three pathogens left can be present, and are able to survive and grow in the product. In practice, they may well cause health problems as a result of consuming cooked potatoes. Therefore, it is important to perform risk assessments for these three pathogens according to literature and expert knowledge.

The results show that the databases used are not complete. *Clostridium botulinum* type B was removed from the list because of its growth characteristics. According to the pathogen database the minimal growth temperature ($T_{\min}$) of *Clostridium botulinum* type B is 12.5°C. However, $T_{\min}$, of

Clostridium botulinum type B, non-proteolytic strains is 5°C (Mitscherlich and Marth, 1984), which is not in the database. The pathogen database does not take differences in proteolytic and non-proteolytic strains into account, yet. The databases therefore have to be extended and updated regularly.

Due to the clear procedure these types of shortcomings are easily detected and corrected.

It is remarkable that *Clostridium botulinum* type A, which was identified as the most relevant pathogen, was not identified in the detailed hazard identification, when using all types of knowledge rules. *Clostridium botulinum* type A was identified in the detailed hazard identification as present on the ingredients, but it was removed from the list by type 3 knowledge rules. The fact is that *Clostridium botulinum* type A is not able to grow in vacuum packed cooked potatoes under normal conditions, in this case at a temperature of 6°C. Its minimum growth temperature was reported to be 10°C (Mitscherlich and Marth, 1984). The reported outbreak of botulism was most probably caused by storage at temperatures higher than 10°C (de Boer, 1996). This shows that the detailed hazard identification, including the use of all knowledge rules, only identifies hazards that are relevant under normal, hygienic conditions.

6.2. Cooked ham

The results of the first two levels of detail applied to cooked ham are shown in Table 6. First a rough hazard identification was performed. For the product cooked ham, the pathogen database only found *Clostridium perfringens* that was reported to have caused problems in the past. After a risk assessment for this pathogen is performed and the risk is estimated to be acceptable, the hazard identification procedure can be continued with a detailed hazard identification based on the potential presence of pathogens in ingredients. The ingredients used in the preparation of cooked ham are ham and brine. Brine consists of salt, water, and several additives, like spices, ascorbate, and glutamate (Brauer, 1987). According to Table 6, 54 pathogens were identified to be present in the ingredients. If knowledge rules were applied type 1 rules resulted in a list of 10 pathogens, type 2 rules in a list of 39 pathogens, and type 3 rules in a list of 14 pathogens (Table 6). To use type 3 rules, it was assumed that the pH of cooked ham is 6.4 ± 0.1, the temperature is 5 ± 1°C, and the water activity is 0.98 ± 0.01, based on data from ICMSF (1988).

If all types of knowledge rules are applied to shorten the list, only four pathogens are left: *Bacillus cereus*, *Bacillus subtilis*, *Clostridium botulinum* type E, and type F. It is sensible to firstly perform risk assessments for these pathogens. However, as mentioned before, selection on growth possibilities is based only on minimum and maximum temperature, pH, and water activity. Inhibitory effects of the nitrite in the brine, which are very important for the safety of cooked ham, are not taken into account. Also, the expert knowledge in the computer program is general expert knowledge, and therefore no specific expert knowledge on bacteria in cooked ham is available. The user needs to have specific knowledge, and based on his experience in the specific situation, the user may not apply all knowledge rules. He may have strong arguments to delete *Bacillus subtilis* or *Bacillus cereus* from the list, or add other pathogens to the list.

Still, the hazard identification procedure identifies hazards that are the most likely to cause problems under normal, hygienic conditions. Therefore the hazard identification procedure may be considered to provide a good start for performing risk assessments for cooked ham.

6.3. Sterilised cow's milk

The last product for which a hazard identification was conducted is sterilised cow's milk (Table 7). The rough hazard analysis did not result in identification of a pathogen that was reported to have caused health problems related to sterilised cow's milk in the past. Continuing with the detailed hazard analysis, 64 pathogens were identified as present on the ingredient raw cow's milk. Application of type 1 rules resulted in identification of zero hazards. This is related to the confirmation of the knowledge rule concerning sterilisation (Table 2), which removed all pathogens. Application of type 2 knowledge rules resulted in a list of 43 pathogens, and application of type 3 rules identified 14 pathogens as hazardous. It was assumed that the pH of milk is 6.5 ± 0.1, that the water activity is 0.98 ± 0.01 (ICMSF, 1988), and that the temperature is 6 ± 1°C (sterilised milk is normally

Table 6
Results of the identification procedure applied to cooked ham and results after application of the three types of knowledge rules

Rough hazard identification	Detailed hazard identification	Knowledge rules			
		Type 1	Type 2	Type 3	Type 1, 2 and 3
Clostridium perfringens	*Acinetobacter spp.*		X		
	Aeromonas spp.			X	
	Aeromonas hydrophila				
	Alcaligenes spp.		X		
	Alcaligenes faecalis				
	Aspergillus flavus		X	X	
	Bacillus spp.	X	X		
	Bacillus anthracis	X		X	
	Bacillus cereus	X	X	X	X
	Bacillus subtilis	X	X	X	X
	Brucella melitensis		X		
	Brucella suis		X		
	Campylobacter spp.		X		
	Campylobacter coli		X		
	Campylobacter jejuni		X		
	Chlamydia psittaci				
	Citrobacter spp.				
	Citrobacter freundii				
	Clostridium spp.	X	X		
	Clostridium botulinum type A	X	X		
	Clostridium botulinum type B	X	X		
	Clostridium botulinum type E	X	X	X	X
	Clostridium botulinum type F	X	X	X	X
	Clostridium perfringens	X	X		
	Corynebacterium spp.		X		
	Enterobacter spp.				
	Enterobacter cloacae				
	Enterobacter hafniae		X		
	Enterococcus spp.		X		
	Erysipelothrix spp.		X		
	Erysipelothrix rhusiopathiae				
	Escherichia spp.		X		
	Escherichia coli		X	X	
	Flavobacterium spp.		X		
	Leptospira spp.				
	Listeria spp.		X		
	Listeria monocytogenes		X	X	
	Moraxella spp.		X		
	Nocardia spp.		X		
	Penicillium spp.		X	X	
	Plesiomonas shigelloides				
	Proteus spp.				
	Pseudomonas spp.		X		
	Pseudomonas aeruginosa			X	
	Salmonella spp.		X	X	
	Salmonella anatum		X		
	Salmonella montevideo		X		
	Serratia spp.		X		
	Serratia liquefaciens				
	Staphylococcus spp.		X		
	Staphylococcus aureus		X	X	
	Streptococcus spp.		X		
	Yersinia spp.		X		
	Yersinia enterocolitica		X	X	

Table 7
Results of the identification procedure applied to sterilised cow's milk and results after application of the three types of knowledge rules

Rough hazard identification	Detailed hazard identification	Knowledge rules			
		Type 1	Type 2	Type 3	Type 2 and 3
no organisms	*Acinetobacter spp.*		X		
were found	*Actinomyces spp.*				
in the database	*Aeromonas spp.*			X	
to have caused	*Aeromonas hydrophila*				
health problems	*Alcaligenes spp.*		X		
related to	*Bacillus spp.*		X		
sterilised cow's milk	*Bacillus cereus*		X	X	X
	Bacillus subtilis		X	X	X
	Brucella spp.			X	
	Brucella abortus		X	X	X
	Brucella melitensis		X	X	X
	Brucella suis		X		
	Campylobacter spp.		X		
	Campylobacter coli		X		
	Campylobacter jejuni		X		
	Chromobacterium spp.		X		
	Citrobacter spp.				
	Clostridium spp.		X		
	Clostridium butyricum		X		
	Clostridium perfringens		X		
	Corynebacterium spp.		X		
	Corynebacterium bovis		X		
	Corynebacterium pyogenes		X		
	Coxiella burnetii				
	Cryptococcus neoformans		X		
	Enterobacter spp.				
	Enterobacter aerogenes				
	Enterobacter cloacae				
	Enterococcus spp.		X		
	Enterococcus faecalis				
	Escherichia spp.		X		
	Escherichia coli		X	X	X
	Flavobacterium spp.		X		
	Leptospira spp.				
	Listeria spp.		X		
	Listeria monocytogenes		X	X	X
	Moraxella spp.		X		
	Mycobacterium spp.		X		
	Mycobacterium bovis				
	Mycobacterium tuberculosis		X		
	Mycoplasma spp.		X		
	Nocardia spp.		X		
	Nocardia asteroides		X		
	Pasteurella multocida		X		
	Proteus spp.				
	Pseudomonas spp.		X		
	Pseudomonas aeruginosa			X	
	Salmonella spp.		X	X	X
	Salmonella dublin		X		
	Salmonella typhi			X	
	Salmonella typhimurium		X		
	Staphylococcus spp.		X	X	X
	Staphylococcus aureus		X	X	X
	Staphylococcus epidermidis		X		
	Streptobacillus moniliformis				
	Streptococcus spp.		X		
	Streptococcus agalactiae				
	Streptococcus bovis				
	Streptococcus dysgalactiae				
	Streptococcus equisimilis				
	Streptococcus pyogenes				
	Streptococcus zooepidemicus		X		
	Yersinia spp.		X		
	Yersinia enterocolitica		X	X	X

cooled after opening of the carton). Combination of the three types of knowledge rules resulted in zero hazards of course, because of the negative result of the application of type 1 rules. Combination of type 2 and type 3 rules however resulted in a list of 10 pathogens. These pathogens are relevant in case the sterilising process is not properly controlled and in case recontamination of milk occurs after sterilisation. The user's knowledge is important to apply this list, which resulted mainly from literature and expert knowledge, for his specific situation.

7. Discussion

A hazard identification procedure was developed and implemented as a computer program, to perform systematically the first step of quantitative risk analysis. The hazard identification procedure was based on the general approach for hazard identification presented by Notermans et al. (1994a). It differs from Notermans' approach by its stepwise identification of important hazards and its interactive character.

Relevant hazards are identified stepwise by the use of several levels of detail. The levels are: rough hazard identification, detailed hazard identification, and comprehensive hazard identification. First, the level of least detail is used to identify the most obvious hazards. For these hazards, risk assessment studies should be performed first. If the calculated risk is acceptable, risk assessments can be carried out for less relevant hazards. Risk assessments should not stop when the most important problems are controlled. As mentioned, risk assessments for less relevant hazards should be performed consecutively.

The interactive character results from the use of several knowledge sources in hazard identification. The knowledge sources are: literature knowledge, expert knowledge and the user's knowledge. By the use of literature knowledge only, theoretical hazards are identified that may not be relevant in certain cases. These theoretical hazards can be treated with relativism by the use of expert knowledge, captured in knowledge rules. Three types of knowledge rules were developed that can be used in combination or apart from each other. The knowledge rules are clearly defined in the hazard identification procedure, and as the defintions are explicit, the knowledge rules may be criticised, and changed if necessary. By the use of knowledge rules, a well founded way is provided to remove theoretical hazards that are not relevant in specific cases. However, expert knowledge is mostly general knowledge, and therefore the user's knowledge is used to focus on those hazards that are most relevant in specific situations. The interactive character of the procedure implies that the procedure does not give definite answers on microbial hazards in food products. The hazard identification procedure is therefore best used by experienced microbiologists, who are supported in their decisions by the best use of literature and expert knowledge. Thus, the most relevant hazards in a product may be assessed efficiently, at three levels of detail.

Implementation of the hazard identification procedure as a computer program resulted in a decision-supporting identification system which uses several databases to identify relevant hazards for certain products. The databases are not complete. This is inevitable, for it is not possible to describe all possible products and ingredients, nor is it possible to describe all existing pathogens, with all related foodborne outbreaks and all related ingredients etc. However, the databases do contain much information to perform reliable hazard identifications. In order to improve hazard identifications in future, the databases should be updated regularly. It is also possible to combine databases, related to quantitative risk analysis, from all over the world. By this combination, much unnecessary work to extend databases can be prevented. This approach may finally result in a generally applicable hazard identification system and a structured method of collection of literature data.

In future, the hazard identification procedure and decision support system will be part of a general procedure for quantitative risk assessments for food products. As well as the hazard identification procedure, the procedure for quantitative risk assessment should be based on the use of three levels of detail and the combination of different knowledge sources.

The hazard identification procedure described above is the first step of a procedure for quantitative risk assessments to be completely developed as a computer-aided system. Currently we are developing

the other steps for quantitative risk assessment and implementing these as computer based systems. These developments will result in a complete decision support system for quantitative risk assessment of microbial contamination of food products.

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