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MKES Tools: a microbial kinetics expert system for developing and assessing food production systems

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SUMMARY

MKES Tools is a microbial kinetics expert system for developing food production systems and assessing product safety. The specific information required as input are: (1) a flowchart of the production system, (2) the factors affecting the survival and growth of food-borne pathogens and (3) the ranges of variation for each factor's parameters. With this information, MKES Tools simulates the growth and survival of pathogenic microorganisms when subjected to many different factor/parameter situations. The responses obtained are then used to estimate the significance of each factor's parameters.

INTRODUCTION

Modern technologies have raised expectations about almost every aspect of our lives. Our attitude towards food safety is no exception. The consumer expects organoleptically perfect ready-to-eat foods with no health risk attached. In order to respond to these demands, the food industry and government are being forced to achieve almost perfect control over and understanding of food products and their production systems.

Microorganisms are associated with every step of food handling, from production to consumption. In order to minimize the risk to the consumer attributed to food-borne pathogens, the Hazard Analysis and Critical Control Point (HACCP) system has been implemented. HACCP was first proposed in 1972 [1], and the basic components of the system have been described by the International Commission on Microbiological Specifications for Food [9]. The HACCP system has been widely adopted for a variety of products and processes including: meat and poultry products [11], retail foods and restaurants [3], chilled foods [4], sous vide [10], canned foods [12], and in the home [2].

One aspect of HACCP which needs improvement is the method of doing a Hazard Analysis. Food products are classified according to several simple rules, and from this analysis appropriate Critical Control Points (CCPs) are determined. Most products now being developed require many controls, some being very subtle and none of them ensuring product safety by themselves. Thus, it is often difficult for experts to reach a consensus regarding the CCPs in a given process [11]. There is a need for improved systems for assessing the potential hazard of new food products and processes.

Agriculture Canada has addressed the problem of modeling food production systems and assessing product safety, and a database and program have been set up on a Lotus spreadsheet. The objectives of the program are to increase understanding of food production systems, provide a basis for scientifically assessing product safety, and finally aid in determining the important factors throughout the production system that affect product safety.

PROGRAM DEVELOPMENT

Flowchart of production system

The first step in modeling a food production system is to provide a flowchart of the production system. The program allows entry of a two dimensional flowchart (Fig. 1). The flowchart units are called areas, and the user simply moves around the screen adding new areas until the flowchart realistically resembles the physical layout of the actual production system.

The example shown in Fig. 1 and in the remaining figures is for demonstration purposes, and no attempt was made to model an existing production system. The example is for a sous vide processed chicken and vegetable dinner. Vegetables are processed, sliced and placed into the container. Raw chicken is mixed with a sauce (Chicken Vat; Fig. 1), then placed on top of the vegetables (Chicken Filler; Fig. 1).

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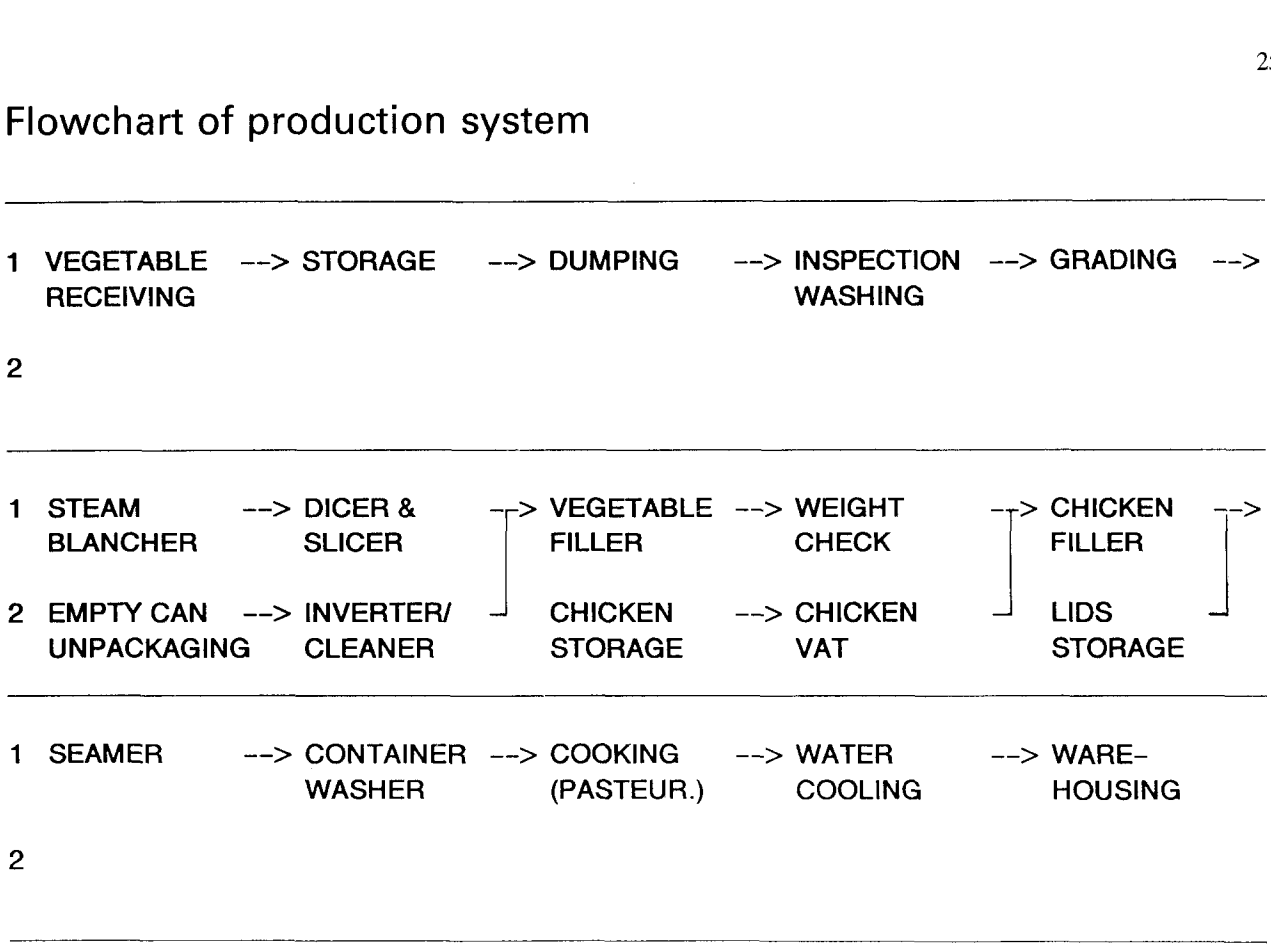

Fig. 1. Example of a hypothetical production system flowchart from Lotus 1,2,3 spreadsheet. Details of the process are given in the text.

The container is covered and hermetically sealed, and heat treated to achieve pasteurization.

Factors affecting product safety

The hazards associated with a product may be chemical or microbiological. In this version of the program, only *Listeria* and *Salmonella* are considered. Other food-borne pathogens such as *Shigella, Aeromonas, Staphylococcus, Escherichia coli* 0157:H7, *Bacillus cereus*, and *Clostridium botulinum* will be added to the program later.

A variety of factors may influence the extent to which a pathogen may constitute a hazard in a particular product. In this model, factors are grouped into five types: contamination; formulation; time/temperature; package permeability; and assembly (Table 1).

Contamination in the present context includes conditions which can either increase or decrease bacterial numbers: initial bacterial load of raw ingredients; contamination by personnel, equipment, and environment; product cleaning, sorting and culling. Formulation and time/temperature indirectly affect the bacterial numbers by controlling the rate of growth or destruction of microorganisms. Formulation includes intrinsic physical or chemical properties of the food such as pH, water activity, (a_w), nitrite, and O_2 levels. Time/temperature specifically includes product cooling (refrigeration and freezing), and heat treatment (pasteurization and sterilization). Package permeability describes the package's moderating affect on the other factors affecting the microbial hazards of the product. Package permeability may be influenced by both the type of packaging and handling. Types of packaging vary from hermetically sealed containers to no packaging at all. This is the most difficult factor to quantify and model mathematically. Finally, assembly allows for the combination of two processing lines into one. The amount of product from each line that will be added as a percentage of the total product determines the intrinsic properties and the levels of microorganisms in the combined product.

In one area, more than one factor can affect the microbial hazards of the product. This version of the program allows the user to identify up to three important factors corresponding to each area of the flowchart (Table 1). This could be increased if necessary in future versions of the program. In the example shown, time/temperature was always an important factor. Contamination was relevant wherever the food or package was handled. Formulation was an appropriate factor in the vegetable receiving and in the chicken storage areas. Assembly was also considered wherever two lines joined. Since packaging includes handling, it was relevant from the time of packaging until unpackaging.

Control of factors within ranges

The program requires detailed descriptions of all the factors affecting the survival and growth of pathogens in the

TABLE 1

Example of Lotus 1,2,3 spreadsheet for entry of factors which affect the extent of microbial hazards in each processing area

Area	Factor		
Vegetable receiving	Formulation	Contamination	Time/temp
Storage	Contamination	Time/temp	
Dumping	Contamination	Time/temp	
Inspection, washing	Contamination	Time/temp	
Grading	Contamination	Time/temp	
Steam blancher	Time/temp		
Dicer & slicer	Contamination	Time/temp	
Empty can unpackag.	Contamination	Time/temp	
Inverter/cleaner	Contamination	Time/temp	
Veg filler assembly	Contamination	Time/temp	
Weight check	Contamination	Time/temp	
Chicken storage	Formulation	Contamination	Time/temp
Chicken vat	Contamination	Time/temp	
Chicken filler	Assembly	Contamination	Time/temp
Lids storage	Contamination	Time/temp	
Seamer	Assembly	Package perm	Time/temp
Container washer	Package perm	Contamination	Time/temp
Pasteur/cooking	Package perm	Time/temp	
Water cooling	Package perm	Contamination	Time/temp
Warehousing	Package perm	Contamination	Time/temp

For each previously designated processing area (Fig. 1), user enters up to three factors which would be expected to influence the extent of microbiological growth and/or contamination.

TABLE 2

Example of Lotus 1,2,3 spreadsheet for entry of factor ranges

Factor # 1	Area Vegetable receiving		Type formulation		
Probability	pH	a_w	Nitrite	O_2	
0.05	5.00E+00	5.00E−01	1.00E+01	1.00E+00	
0.9	6.00E+00	2.00E+00	5.00E+01	1.00E+00	
0.05	7.00E+00	4.50E+00	1.00E+02	1.00E+00	
Factor # 7	Area Dicer & slicer		Type contamination		
Probability	*Salmonella*	*Listeria*	*Shigella*	*Aeromonas*	*S. aureus*
0.05	1.00E+00	1.01E+01	0.00E+00	0.00E+00	0.00E+00
0.9	1.00E+01	1.05E+01	0.00E+00	0.00E+00	0.00E+00
0.05	1.00E+02	1.50E+01	0.00E+00	0.00E+00	0.00E+00

For each of the previously designated factors from Table 2, the user enters appropriate numerical ranges for the various parameters within each factor.

product. An example of two hypothetical factor descriptions is shown in Table 2. Each record of the input database corresponds to a specific factor. Already included in each record are the area where the factor occurs, and the factor type. Depending on the factor, the user is required to specify ranges for appropriate numerical parameters. For consistency, the minimum and maximum of each range should be specified such that it has a 5% chance of occurring. The mean value for each parameter is also required.

In this version of the model, a total of 62 parameters over all processing areas can be entered as ranges. Additional parameters must be entered as constants. This is done by making the minimum, maximum, and mean values all the same.

TABLE 3

Output from Lotus 1,2,3 spreadsheet describing accumulation of *Salmonella* and *Listeria* in response to an example of factor combinations described in one event

Area	Factor	Type	Level
Warehousing	Time/temp	Time	2
Warehousing	Time/temp	Temp	10
Pasteur/cooking	Time/temp	Time	0.06
Pasteur/cooking	Time/temp	Temp	60
Warehousing	Contamination	*Salmonella*	100
Lids storage	Contamination	*Salmonella*	1.5
Chicken filler	Contamination	*Salmonella*	1.5
Vegetable receiving	Formulation	a_w	1.5

Main processing line

Type	VEGE	STOR	DUMP	INSP	GRAD	STEA	→
Salmonella	1.00E+03	4.14E+03	1.07E+04	2.42E+04	5.14E+04	1.04E+05	
Listeria	1.05E+01	3.23E+01	7.76E+01	1.69E+02	3.54E+02	7.18E+02	
→	DICE	VE.F	WEIG	CH.F	SEAM	CONT	→
Salmonella	1.03E+05	1.25E+05	2.52E+05	3.55E+05	4.99E+05	1.00E+06	
Listeria	7.18E+02	8.79E+02	1.78E+03	2.52E+03	3.55E+03	7.13E+03	
→	PAST	WATE	WARE	FINA			
Salmonella	2.00E+06	1.51E+06	3.03E+06	6.06E+06			
Listeria	1.43E+04	1.08E+04	2.17E+04	4.34E+04			

For each combination of all the factors (one event), microbial cell numbers accumulation is shown as the product moves from the receiving area to the consumer.

Events

The factors described in the factors database constitute a model of the production system. By simultaneously varying the parameter levels of these factors, it is possible to simulate a total of 256 events that could occur. The program allows either a full factorial experiment if less than eight factors are to be varied, or a fractional factorial experiment [7] for more than eight factors.

For each event, an accumulation is made to obtain the bacterial counts in the final product (Table 3). The growth equations used in this model are taken from a pathogen modeling program developed by Buchanan and coworkers [5,6]. Arrhenius equations for thermal inactivation of pathogens used in the present study have been described by Hallström [8].

For each event, the levels of each factor type previously designated as variable can be displayed (Table 3). The accumulation of each microorganism as the product moves from the receiving area to the consumer may be shown. The accumulation at each step of the production system can be graphically displayed to complement the numerical results (Fig. 2).

Sensitivity analysis

Based on the final bacterial counts (responses), an analysis of variance is performed to determine the significance of each parameter on the response variable. It must be remembered that the sensitivity of the process to a parameter is directly dependant on the range over which that parameter was varied. Therefore all significant effects must be viewed in this context.

The sensitivity analysis of the fractional factorial experiment determines the relative significance of all the parameters as shown in Table 4. For the current example, the eight most significant parameters in descending order were: warehousing time and temperature, pasteurization time and temperature, warehousing contamination (*Salmonella*), lids storage contamination (*Salmonella*), chicken filler contamination (*Salmonella*), and vegetable receiving a_w.

The sensitivity analysis of the full factorial experiment (where only the eight most significant parameters are varied) is much more accurate, and provides details of interactions between parameters. Sums of squares are presented as an indication of the relative importance of main effects and interactions between parameters (Table 5). For the present example, it was confirmed that the time of warehousing (A) was most important with the temperature of warehousing (B) rated second. Pasteurization time (C) and temperature (D) were next in relative significance. Contamination in warehousing (E) was almost as important as the pasteurization time and temperature. Water activity of the raw vegetables (H) was a significant parameter, but this seemed to be

TABLE 4

Output of general sensitivity analysis from Lotus 1,2,3 spreadsheet

Area	Factor	Parameter	L–Range–H		S–Sens–L	
Vegetable receiving	Formulation	pH	5	7	6.0	187.5
Vegetable receiving	Formulation	a_w	0.985	0.995	10.7	47.0
Vegetable receiving	Formulation	Nitrite	10	100	0.3	26.3
Vegetable receiving	Contamination	*Salmonella*	100	10000	1.4	0.0
Vegetable receiving	Contamination	*Listeria*	10.05	15	0.1	0.1
Storage	Time/temp	Time	2	4	0.0	0.0
Storage	Time/temp	Temp	10	20	0.0	0.1
Dumping	Contamination	*Salmonella*	2050	2500	0.0	0.0
Dumping	Contamination	*Listeria*	5.05	15	0.0	0.6
Inspection, washing	Contamination	*Salmonella*	2050	2500	0.0	0.0
Inspection, washing	Contamination	*Listeria*	5.05	15	0.0	0.0
Grading	Contamination	*Salmonella*	2050	2500	0.0	0.0
Grading	Contamination	*Listeria*	10.05	15	0.0	0.0
Steam blancher	Time/temp	Time	0.001	0.1	0.1	0.0
Steam blancher	Time/temp	Temp	50	60	0.0	0.0
Empty can unpack	Contamination	*Salmonella*	1.5	200	0.0	0.0
Empty can unpack	Contamination	*Listeria*	10.05	15	0.0	0.0
Dicer & slicer	Contamination	*Salmonella*	1	100	0.1	0.1
Dicer & slicer	Contamination	*Listeria*	10.05	15	0.0	0.0
Inverter/cleaner	Contamination	*Salmonella*	1.5	200	0.0	0.4
Inverter/cleaner	Contamination	*Listeria*	10.05	15	0.0	0.0
Vegetable filler	Assembly	%Line2	0.5	0.3	0.4	0.2
Vegetable filler	Contamination	*Salmonella*	1.5	200	0.0	20.2
Vegetable filler	Contamination	*Listeria*	10.05	15	0.0	0.8
Chicken storage	Formulation	a_w	0.985	0.995	0.0	0.2
Chicken storage	Contamination	*Salmonella*	1.5	3000	0.0	0.2
Chicken storage	Contamination	*Listeria*	10.05	15	0.0	0.0
Chicken storage	Time/temp	Time	2	4	0.1	0.1
Chicken storage	Time/temp	Temp	10	20	0.0	0.0
Weight check	Contamination	*Salmonella*	1.5	3000	0.0	0.0
Weight check	Contamination	*Listeria*	10.05	15	0.0	0.0
Chicken vat	Contamination	*Salmonella*	20	400	0.1	0.0
Chicken vat	Contamination	*Listeria*	15	100	0.0	0.0
Chicken filler	Assembly	%Line2	0.4	0.2	0.0	0.0
Chicken filler	Contamination	*Salmonella*	1.5	3000	11.0	1.0
Chicken filler	Contamination	*Listeria*	10.05	15	0.1	0.4
Lids storage	Contamination	*Salmonella*	1.5	3000	15.0	16.1
Lids storage	Contamination	*Listeria*	10.05	15	0.5	1.0
Seamer	Assembly	%Line2	0.4	0.2	0.1	21.2
Container washer	Contamination	*Salmonella*	1.5	3000	0.5	0.0
Container washer	Contamination	*Listeria*	10.05	15	0.1	0.0
Pasteur/cooking	Time/temp	Time	0.06	6	92.3	59.0
Pasteur/cooking	Time/temp	Temp	60	80	91.6	63.9
Water cooling	Contamination	*Salmonella*	1.5	300	3.9	0.1
Water cooling	Contamination	*Listeria*	10.05	15	0.0	0.0
Warehousing	Contamination	*Salmonella*	100	10000	44.7	0.2
Warehousing	Contamination	*Listeria*	10.05	15	0.5	0.0
Warehousing	Time/temp	Time	2	200	493.8	1578.7
Warehousing	Time/temp	Temp	10	20	261.0	68.3

L–Range–H; low and high values for the range.
S–Sens–L; sensitivity analysis for *Salmonella* and *Listeria*.

TABLE 5

Output of detailed sensitivity analysis from Lotus 1,2,3 spreadsheet

Hazard: *Salmonella*

Main effects:							
A	595.38	C	89.07	E	50.37	G	0.00
B	220.07	D	89.07	F	0.00	H	24.96
Second order interactions:							
AB	218.12	BC	8.95	CE	5.58	DH	0.03
AC	12.06	BD	8.95	CF	0.00	EF	0.00
AD	12.06	BE	5.57	CG	0.00	EG	0.00
AE	5.75	BF	0.00	CH	0.03	EH	0.01
AF	0.00	BG	0.00	DE	5.58	FG	0.00
AG	0.00	BH	22.82	DF	0.00	FH	0.00
AH	21.37	CD	89.07	DG	0.00	GH	0.00

Legend:

A Ware	Time/temp	Time	E Ware	Contamination	*Salmonella*
B Ware	Time/temp	Temp	F Lids	Contamination	*Salmonella*
C Past	Time/Temp	Time	G Ch.F	Contamination	*Salmonella*
D Past	Time/Temp	Temp	H Vege	Formulation	a_w

This example gives the sums of squares for main effects and second order interactions for all parameters which were entered as variables. All other parameters were set at their mean level or entered as constants.

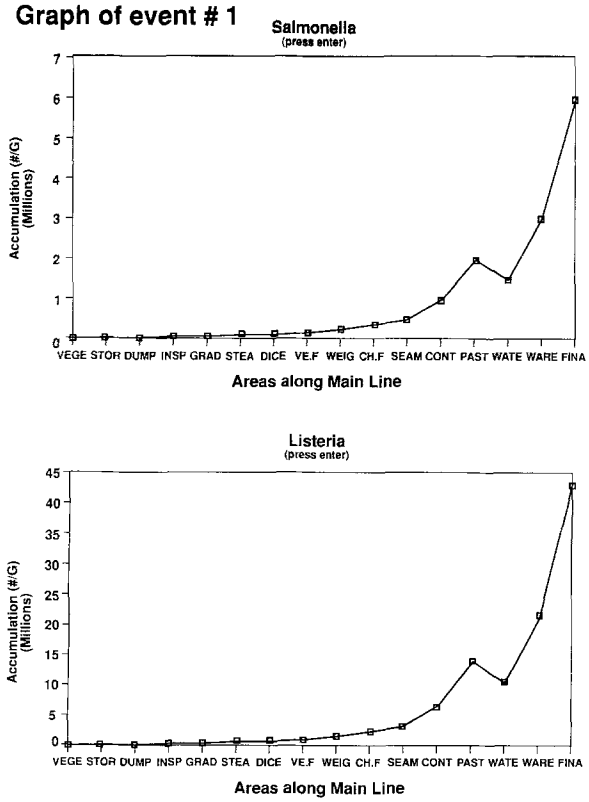

Fig. 2. Lotus 1,2,3 plot of microbial cell numbers from a hypothetical production system using data from Table 3.

largely due to its interaction with the warehousing time (AH) and temperature (BH). Contamination in lids storage (F) and in chicken filling (G) were considered unimportant. The interaction between the time and temperature in warehousing (AB) was very high, as was the interaction between the pasteurization time and temperature (CD).

The sensitivity analysis is important since the parameters having the greatest impact on final contamination levels will suggest possible critical control points. The sensitivity analysis can also be used to assess and improve the model, since it allows identification of unrealistic results. More specifically, the model should reinforce an expert's opinion, otherwise the model and/or the opinion should be questioned.

CONCLUSIONS

The program was designed in its present form in order to demonstrate the potential value of such a model in developing safe production systems. Considerable additional work will be required to: incorporate other pathogens and chemical hazards into the model; improve the thermal inactivation equations; improve the modeling of factors such as contamination, packaging and assembly; include factors to allow for competition between indigenous microflora; and produce a user-friendly version.

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