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Journal of FOOD PROCESSING and PRESERVATION

Edited by
T.P. LABUZA

FOOD & NUTRITION PRESS, INC.
WESTPORT, CONNECTICUT 06880
USA

VOLUME 6, NUMBER 1 MARCH 1982

JOURNAL OF FOOD PROCESSING AND PRESERVATION

All articles for publication and inquiries regarding publication should be sent to Prof. T. P. Labuza, University of Minnesota, Department of Food Service and Nutrition, St. Paul, MN 55108 USA.

All subscriptions and inquiries regarding subscriptions should be sent to Food & Nutrition Press, Inc., 265 Post Road West, Westport, Connecticut USA.

One volume of four issues will be published annually. The price for Volume 6 is $60.00 which includes postage to U.S., Canada, and Mexico. Subscriptions to other countries are $72.00 per year via surface mail, and $80.00 per year via airmail.

Subscriptions for individuals for their own personal use are $40.00 for Volume 6 which includes postage to U.S., Canada, and Mexico. Personal subscriptions to other countries are $52.00 per year via surface mail, and $60.00 per year via airmail. Subscriptions for individuals should be sent direct to the publisher and marked for personal use.

The *Journal of Food Processing and Preservation* is listed in *Current Contents/Agriculture, Biology & Environmental Sciences (CC/AB).*

The *Journal of Food Processing and Preservation* (USPS 456-470) is published quarterly by Food & Nutrition Press, Inc. — Office of publication is 265 Post Road West, Westport, Connecticut 06880 USA.

Second class postage paid at Westport, CT 06880.

POSTMASTER: Send address changes to Food & Nutrition Press, Inc., 265 Post Road West, Westport, CT 06880.

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PRESERVATION

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WESTPORT, CONNECTICUT 06880 USA

Food & Nutrition Press, Inc.
Westport, Connecticut 06880 USA

ISSN: 0149-6085

Printed in the United States of America

CONTENTS

PRODUCTION OPTIMIZATION[1]

DAVID R. THOMPSON

Agricultural Engineering Department
and Department of Food Science and Nutrition
University of Minnesota
St. Paul, Minnesota

Received for Publication September 10, 1981

INTRODUCTION

Experimentation as known to most scientific researchers is restricted to the research lab and the pilot plant. Once a product or procedure is adopted for production, conventional experimentation is not possible. However, it can be argued that production plants should test controllable variations in order to improve the operation. To be effective these controlled variations must satisfy several production characteristics.

The first characteristic is that the experimental procedure must seek the optimum response. Although a production plant is carefully scaled up from a pilot plant operation, there is usually distortion in the scale up process. Therefore the production plant does not operate at the absolute optimum. Furthermore the optimum operational parameters will tend to drift with time. Consequently, the controlled variation of the production plant must be designed to seek the optimum operational characteristics rather than rigidly adhering to the specified operational parameters.

The second characteristic is related to the goals set for production. Controlled variation of process must neither significantly decrease the production rate nor cause quality control or safety problems.

The third characteristic of production optimization is that the sample sizes are very large. Frequently the response variable is measured over a period of one or more days. The experimental results may represent the total population rather than a sample drawn from the population.

The fourth important characteristic of production optimization is that it is controlled by people skilled in management and production, not scientists or statisticians. Consequently the implementation

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[1]Paper No. 11886 Scientific Journal Series, Agricultural Experiment Station, University of Minnesota, St. Paul, Minnesota 55108

must require little time, the statistical analysis and interpretation should be simple, and a management committee (which may include a researcher or statistician) should periodically review the results of the production optimization program. Normally the production optimization should proceed automatically until this management or review committee recommends changes.

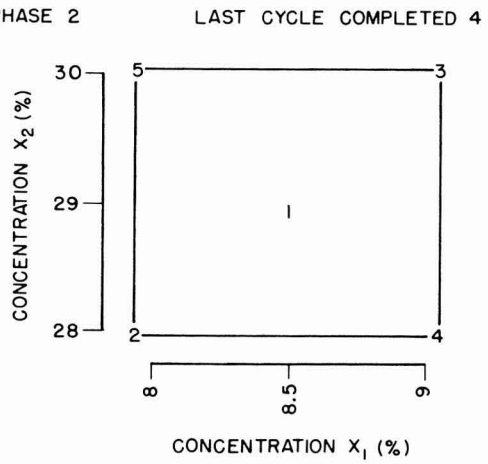
Several steps must be taken before a production optimization program is started. If the current production operation is unacceptable, it may be best to stop production while a few screening experiments are undertaken either in the production facility or in the pilot plant in order to improve production to an acceptable level. Only if the current production operation is acceptable, should a production optimization program be undertaken.

When production optimization is undertaken, quality and safety constraints and the objective or response function that is to be optimized are identified. Both constraints and the response function must be measurable quantities and the acceptable limits for each constraint must be specified. The independent variables, those variables which will be systemtically varied in search of the optimal operation, must be selected. An appropriate step size is specified for each independent variable. A single step in any independent variable should produce a small but measurable change in the response variable. However, this step size must be sufficiently small to assure predictable changes in the quality control and safety constraints.

Several production optimization procedures have been proposed. One of the first procedures (Box 1957) was called evolutionary operation. Some authors use evolutionary operation as a synonym for production optimization, but in this paper evolutionary operation will refer only to the specific procedure. A simplex procedure, Spendley *et al.* (1962), for production optimization is also reviewed in this paper.

EVOLUTIONARY OPERATION

Evolutionary operation (Box 1957 and Box and Draper 1969) for practical purposes is restricted to two or three independent variables. The authors recommend that an evolutionary operation information board (Fig. 1) be posted in the production plant. The phase number at the top of this indicates the number of permanent modifications in the independent variables made since these variables were selected in accordance with the current objective or response function and constraints. The last cycle completed shows the number



		OBJECTIVE Yield (%) Maximize		CONSTRAINTS Texture Between 2 and 3	
		80.5	80.3	2.45	2.75
Running Averages		79.5		2.55	
		79.3	79.3	2.43	2.65
Error Limits	$\frac{2s}{\sqrt{n}}$	0.8		0.15	
Effects	x_1	-0.1 ± 0.8		0.26 ± 0.15	
with	x_2	1.1 ± 0.8		0.12 ± 0.15	
2s	x_1x_2	-0.1 ± 0.8		0.04 ± 0.15	
Limits	mean	0.3 ± 0.7		0.02 ± 0.13	

FIG. 1. EVOLUTIONARY OPERATION INFORMATION BOARD

of times there has been a production run for all of the design points in the current phase.

The diagram (Fig. 1) then specifies the current phase design, for example, the independent variables are concentrations of ingredients x_1 and x_2 as expressed in percent. The range in which each of these is to be studied in the current phase is identified on the appropriate axis. In other words the concentration of x_1 is to be varied between 8 and 9% and the concentration of x_2 between 28 and 30%. The specific design points and their order of consideration within each cycle is indicated in the box. In this example, for the

first production run and the concentration of x_1 and x_2 are 8.5 and 29%, respectively. This is the center point of the design. The next production run within a cycle will have x_1 and x_2 at 8 and 28%. The production runs follow the order of the numbers in the box.

The next lines of the information board indicate the objective or response function and constraints. In the example, there is only one constraint, texture. In this section the range for the constraints is also provided.

Sample data (Table 1) based on the optimization design (Fig. 1) lists the yield (response function) and texture (constraint) for four completed cycles. The response and texture values at each design point are totalled after each completed cycle. The total sum of the values and the sum of values squared are also recorded for computation of a standard deviation. This information is used to calculate the numbers for the lower part of the information board.

The equations (Table 2) determine the running averages, standard deviations and variable effects. The running averages at each design point are recorded in the same relative location as the run numbers listed in the upper part of the information board.

Table 1. Evolutionary operation data

Conditions	Yield							
Cycle/sum	1	2	3	4	5	Σy	Σy^2	s
1	79.2	79.4	81.1	79.1	80.2			
sum	79.2	79.4	81.1	79.1	80.2	399	31843	0.85
2	79.7	78.6	80.4	78.5	80.6			
sum	158.9	158.0	161.5	157.6	160.8	797	63496	0.87
3	80.1	79.2	79.6	80.1	81.4			
sum	239.0	237.2	241.1	237.7	242.2	1197	95563	0.85
4	78.9	79.8	80.1	79.6	79.6			
sum	317.9	317.0	321.2	317.3	321.8	1595	127244	0.76
Conditions	Texture							
Cycle/sum	1	2	3	4	5	Σy	Σy^2	s
1	2.5	2.3	2.8	2.7	2.5			
sum	2.5	2.3	2.8	2.7	2.5	12.8	32.9	0.19
2	2.6	2.6	2.7	2.7	2.4			
sum	5.1	4.9	5.5	5.4	4.9	25.8	66.8	0.15
3	2.6	2.5	2.7	2.6	2.4			
sum	7.7	7.4	8.2	8.0	7.3	38.6	99.6	0.14
4	2.5	2.3	2.8	2.6	2.5			
sum	10.2	9.7	11.0	10.6	9.8	51.3	132	0.15

Table 2. Equations for evolutionary operation for two independent variables

Term	Equation
Response mean	$\bar{y}_i = \frac{\sum_{j=1}^{n} y_{ij}}{n}$
Standard deviation	$s = \left[\frac{\sum_{i=1}^{5} \sum_{j=1}^{n} (y_{ij}^2) - \frac{\left(\sum_{i=1}^{5} \sum_{j=1}^{n} y_{ij} \right)^2}{5n}}{(5n-1)} \right]^{1/2}$
2s error limits	$\pm \frac{2s}{\sqrt{n}}$
x_1 effect	$(\bar{y}_3 + \bar{y}_4 - \bar{y}_2 - \bar{y}_5)/2$
x_2 effect	$(\bar{y}_3 + \bar{y}_5 - \bar{y}_2 - \bar{y}_4)/2$
x_1x_2 effect	$(\bar{y}_2 + \bar{y}_3 - \bar{y}_4 - \bar{y}_5)/2$
Change in mean	$(\bar{y}_2 + \bar{y}_3 + \bar{y}_4 + \bar{y}_5 - 4\bar{y}_1)/5$
Change in mean 2s error limit	$\pm \frac{4s}{\sqrt{5n}} = \pm 0.89 * \frac{2s}{\sqrt{n}}$

The standard deviation uses measurements at all of the experimental locations for every cycle. An error limit based on twice the standard deviation divided by the square root of the number of cycles indicates the error limits is an approximation of the student t values for the 95% confidence limits. When n is small, the value should be greater than 2 and when n is large the value should be slightly less than 2. However, Box (1957), recommends the use of 2 for simplifying the calculations.

The main effects and the interactive effect can be calculated with the equations (Table 2) or these equations can be easily visualized from the figure on the information board. In the example (Fig. 1) the effect of increasing x_2 appears to be significant since it is larger than the error limit. Both the interaction and x_1 effects are slightly negative but insignificant. The calculated constraint effects indicate that an increase in x_2 may increase the texture value, but x_2 could probably be increased several percent before the texture constraint

exceeded its upper limit. These effect calculations suggest that a permanent increase in the concentration of x_2 and the initiation of phase three is probably justified.

The final line (Fig. 1) indicates the effect of the evolutionary operation relative to the center operational point. This effect is calculated by the appropriate equation (Table 2) and its error limit is 0.89 times the error limit on the preceding effects (Table 2). In the example the use of evolutionary operation is effectively increasing the yield by 0.3 ± 0.7 percent versus a constant operation. The lack of a significant increase or decrease indicates a nearly linear response in the selected ranges of the independent variables.

Box (1957) suggested adding an additional set of information at the bottom of the information board. The additional information indicates the accuracy of the error limit estimation. This involves Chi square values and calculations which are probably beyond the capacity of most production personnel.

The calculations for evolutionary operation can be simplified by estimating the standard deviation from the range rather than the sums and squares of responses.

Weighting factors (Table 3) are used to calculate the standard deviation from the range of values. The standard deviation is calculated by multiplying the range of the responses by the weighting factor, w_n, corresponding to the number of responses listed in this table. This can be applied directly, but Box and Draper (1969) recommend basing the standard deviation on the differences between the response levels for the most recent cycle and the

Table 3. Calculating the standard deviation from the range of responses[1]

Number of Responses n	Weighing Factor w_n	Number of Responses n	Weighing Factor w_n
2	0.8862	12	0.3069
3	0.5908	13	0.2998
4	0.4857	14	0.2935
5	0.4299	15	0.2880
6	0.3946	16	0.2831
7	0.3698	17	0.2787
8	0.3512	18	0.2747
9	0.3367	19	0.2711
10	0.3249	20	0.2677
11	0.3152		

[1] $s = w_n *$ (range of responses)

average responses for the preceding cycles. The application of the weighting factors for calculating the standard deviation from the yield data (Table 1) is illustrated in Table 4. In this table the difference line is always the result from the latest cycle subtracted from the previous mean. Because the difference is between the mean and a single measurement, the standard deviation calculation must be weighted by the factor $[(n-1)/n]^{0.5}$ where n is the number of cycles completed.

Table 4. Calculating the standard deviation from the yield data

Conditions Cycle/sum	1	2	3	4	5	s*
1	79.2	79.4	81.1	79.1	80.2	
mean	79.2	79.4	81.1	79.1	80.2	
2	79.7	78.6	80.4	78.5	80.6	
difference	-0.5	0.8	0.7	0.6	-0.4	0.40
sum	158.9	158.0	161.5	157.6	160.8	
mean	79.5	79.0	80.8	78.8	80.4	
3	80.1	79.2	79.6	80.1	81.4	
difference	-0.6	-0.2	1.2	-1.3	-1.0	0.88
sum	239.0	237.2	241.1	237.7	242.2	
mean	79.7	79.1	80.4	79.2	80.7	
4	78.9	79.8	80.1	79.6	79.6	
difference	0.8	-0.7	0.3	-0.4	1.1	0.67
sum	317.9	317.0	321.2	317.3	321.8	
mean	79.5	79.3	80.3	79.3	80.5	

*$s = \left(\frac{n-1}{n}\right)^{1/2} w_n R_n$

Evolutionary operation can be generalized to three independent variables (Fig. 2). Two production runs, numbers five and ten are located at the overall center of the design space. The equations for calculating the entries for the information board when there are three independent variables are listed in Table 5. Extension of evolutionary operation to more than three independent variables is possible but awkward.

SIMPLEX DESIGN FOR PRODUCTION OPTIMIZATION

Simplicity is one objective for effective production optimization designs. While the evolutionary operation procedure is less complex than most laboratory experiments, it does involve significant

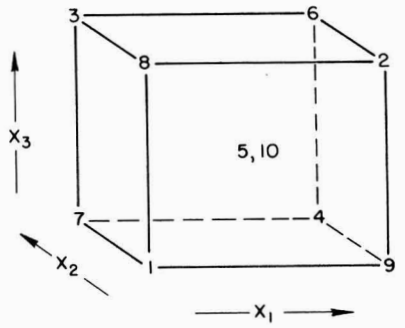


FIG. 2. EVOLUTIONARY OPERATION WITH THREE INDEPENDENT VARIABLES

Table 5. Evolutionary operation equations for three independent variables

Term	Equation
Response mean	$\bar{y}_i = \dfrac{\sum_{j=1}^{n} y_{ij}}{n}$
Standard deviation	$S = \left[\dfrac{\sum_{i=1}^{10} \sum_{j=1}^{n} (y_{ij}^2) - \left(\sum_{i=1}^{10} \sum_{j=1}^{n} y_{ij} \right)^2 \Big/ 10n}{(10n - 1)} \right]^{1/2}$
2s Error Limits	$\pm \dfrac{2s}{\sqrt{2n}}$
x_1 effect	$(\bar{y}_2 + \bar{y}_4 + \bar{y}_6 + \bar{y}_9 - \bar{y}_1 - \bar{y}_3 - \bar{y}_7 - \bar{y}_8)/4$
x_2 effect	$(\bar{y}_3 + \bar{y}_4 + \bar{y}_6 + \bar{y}_7 - \bar{y}_1 - \bar{y}_2 - \bar{y}_8 - \bar{y}_9)/4$
x_3 effect	$(\bar{y}_2 + \bar{y}_3 + \bar{y}_6 + \bar{y}_8 - \bar{y}_1 - \bar{y}_4 - \bar{y}_7 - \bar{y}_9)/4$
x_1x_2 effect	$(\bar{y}_1 + \bar{y}_4 + \bar{y}_6 + \bar{y}_8 - \bar{y}_2 - \bar{y}_3 - \bar{y}_7 - \bar{y}_9)/4$
x_1x_3 effect	$(\bar{y}_1 + \bar{y}_2 + \bar{y}_6 + \bar{y}_7 - \bar{y}_3 - \bar{y}_4 - \bar{y}_8 - \bar{y}_9)/4$
x_2x_3 effect	$(\bar{y}_1 + \bar{y}_3 + \bar{y}_6 + \bar{y}_9 - \bar{y}_2 - \bar{y}_4 - \bar{y}_7 - \bar{y}_8)/4$
$x_1x_2x_3$ effect	$(\bar{y}_6 + \bar{y}_7 + \bar{y}_8 + \bar{y}_9 - \bar{y}_1 - \bar{y}_2 - \bar{y}_3 - \bar{y}_4)/4$
Change in mean	$[\bar{y}_1 + \bar{y}_2 + \bar{y}_3 + \bar{y}_4 + \bar{y}_6 + \bar{y}_7 + \bar{y}_8 + \bar{y}_9 - 4(\bar{y}_5 + \bar{y}_{10})]/10$ with an error limit of $\pm 0.632 \dfrac{2s}{\sqrt{n}}$

statistical calculations. It also requires replicated experiments, which may or may not be desirable. The simplex design for production optimization (Spendley *et al.* 1962) avoids these criticisms.

The simplex design is based on a geometric figure in k dimensions involving k + 1 experimental points where k is the number of independent variables. If there are two independent variables, the simplex design is an equilateral triangle. With three independent variables the simplex design is a tetrahedron. Production optimization with a simplex design first requires a production run at each design location in the original simplex. Then without replicating any of the production runs, a new point is added to the design, thereby generating a new simplex. Upon completing the additional production run, another move is possible. Thus, with simplex designs there is a change in production specifications after every production run (excluding the first k production runs).

Simplex designs are efficient procedures for following slopes to the optimum response (Spendley *et al.* 1962). The general direction of the optimum is normal to the face of the figure opposite the vertex with the least optimal response. The simplex production optimization scheme accomplishes this movement by forming a mirror immage of the vertex with the least optimum response (Fig. 3).

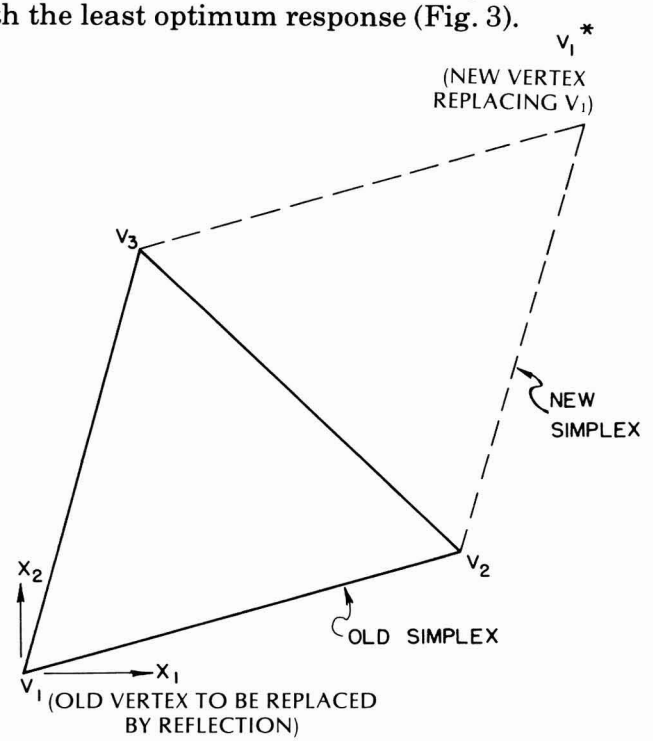


FIG. 3. THE REFLECTION OF THE VERTEX WITH THE LEAST OPTIMUM RESPONSE IN SIMPLEX PRODUCTION OPTIMIZATION

Table 6. Simplex optimization design

Vertex	Independent Variable Coordinates
v_1	0, 0, 0, . . ., 0
v_2	p, q, q, . . ., q
v_3	q, p, q, . . ., q
.	.
.	.
.	.
v_k	q, q, . . ., q, p, q
v_{k+1}	q, q, . . ., q, q, p

where

$$p = \frac{1}{k\sqrt{2}} \left[(k-1) + \sqrt{k+1}\ \right]$$

$$q = \frac{1}{k\sqrt{2}} \left(\sqrt{k+1} - 1\right)$$

TABLE 7. Values of p and q for simplex designs

k	p	q
2	0.97	0.26
3	0.94	0.24
4	0.93	0.22
5	0.91	0.20
6	0.90	0.19

The formal procedure (Tables 6 and 7) establishes the vertices or locations of the production runs in a simplex design. Each row gives the k scaled coordinates for a particular vertex. This design assumes that each independent variable has been scaled so that its starting point is zero and the step size is 1.0. In practice it is best to transform the basic design into the operational levels for each independent variable (Table 8). Table 9 illustrates a three variable design.

After production runs are completed for each vertex in the original simplex design, the vertex which generated the least optimal response, v_j, is replaced by its reflected image, v_j^*. This is done by subtracting the value of the vertice being reflected from twice the mean of the remaining vertices.

$$v_j^* = \frac{2}{k} (v_1 + v_2 + \ldots + v_{j-1} + v_{j+1} + \ldots v_{k+1}) - v_j \qquad (1)$$

Table 8. An application of simplex design to the problem in Fig. 1

Characteristic	x_1(%)	x_2(%)
Base level	8.5	29
Step increase	0.5	1.0
Transformation equation (code, c, to actual level)	0.5c + 8.5	c + 29
Coded design:		
v_1	0	0
v_2	0.97	0.26
v_3	0.26	0.97
Actual design:		
v_1	8.5	29
v_2	8.99	29.26
v_3	8.63	29.97

Table 9. A simplex design for a three variable problem

Characteristic	x_1	x_2	x_3
Variable	process time	process temp.	salt level
Units	min.	°C	%
Base level	5	80	15
Step increase	0.25	5	1
Transformation equation (code, c, to actual)	0.25c + 5	5c + 80	c + 15
Coded design:			
v_1	0	0	0
v_2	0.94	0.24	0.24
v_3	0.24	0.94	0.24
v_4	0.24	0.24	0.94
Actual design:			
v_1	5	80	15
v_2	5.235	81.2	15.24
v_3	5.06	84.7	15.24
v_4	5.06	81.2	15.94

Application of a simplex design requires three rules (Spendley *et al.* 1962).

1. Ascertain the least optimum response generated by any experimental run in the current simplex and replace the vertex at which this least optimum value occurred with its reflected image according to Eq. 1.

2. If a vertex in the simplex remains unchanged through k + 2 successive simplexes, repeat the production run at that vertex before generating a new vertex. Because of experimental errors it is possible that a spuriously high (low) result may be generated. This would anchor the simplex to the vertex as though it were a true optimum. Rule 2 specifies repeating the measure at a point that appears to be the optimum to verify whether it is an optimum or an erroneous result. This also accommodates a drifting optimum.
3. If the response at the new vertex is the least optimum in the simplex, do not apply rule 1 as this would reflect the vertex back to its previous position. Generate a new simplex by applying Eq. 1 to the vertex with the second least optimal value. When the new vertex is the lowest reading in the simplex, the simplex is probably straddling a ridge of the response function. By reflecting the second lowest value, the simplex is re-oriented and therefore has a better chance of climbing the ridge line. This also forces the simplex to circle about an optimum rather than oscillating over limited range.

The three rules can be summarized briefly as: move by rejecting the least optimum observation unless (1) another observation is too old—in which case renew the latter or (2) a move would cause a return to the previous simplex—in which case try the next most favorable direction.

CONCLUSIONS

The simplex procedure involves no statistics and is very simple to apply. It is much more likely to make erroneous moves than the evolutionary operation procedure. However, it quickly corrects erroneous moves and therefore is probably more effective. Another advantage is the ability to handle four or more independent variables. Normally no more than three independent variables are studied in production optimization because of the complexity, but this design may be used for experiments in non-production environments.

Spendley *et al.* (1962) did not recommend a formal procedure for satisfying the constraint conditions, but the observed level of one or more constraint variables may require variation from the three recommended rules.

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NOTATION

Symbol	Definition
c	Coded level from a simplex design.
k	The number of independent variables in the optimization design.
n	The number of cycles of evolutionary operation completed.
p,q	Coded variable levels for the simplex design.
R_n	Range of differences after m cycles.
s	Standard deviation.
t	The student t value based on the degrees of freedom and probablity level.
w_n	Weighting factor for finding the standard deviation from the range of n responses.
v_j	A vertice in a simplex design. In equation one, v_j represents the vertice with the lowest recorded response.
v_j^*	A new vertice to replace v_j.
x_i	An independent variable.
$\overline{y_i}$	The mean of all responses during the phase at design location i.
y_{ij}	The response level in cycle j for design location i.

Greek

Σ	Summation

A COMPARISON OF STIRRED CURD CHEESES MADE FROM NORMAL MILKS AND RECONSTITUTED NONFAT DRY MILK WITH VEGETABLE OILS[1]

M. O. MOHAMED, H. A. MORRIS and W. M. BREENE

Department of Food Science and Nutrition
University of Minnesota
St. Paul, Minnesota 55108

Received for Publication November 6, 1981

ABSTRACT

Cheeses made from 30% total solids (TS) mixtures of reconstituted nonfat dry milk and vegetable oils were compared with cheeses made from 12.5% TS mixtures and with cheeses made from normal milk. The 30% TS filled milks produced cheeses having firmer curd, lower moisture content and better body and texture characteristics than those made from 12.5% TS filled milks. The rate of acid development during cheese making was slower in filled milks than in normal milks. Filled cheeses were always graded lower than normal milk cheeses. The 30% TS filled milk cheeses were significantly harder than 12.5% TS filled milk cheeses and normal milk cheeses. Cheeses containing coconut oil were harder than those containing soybean oil.

INTRODUCTION

Cheese production in underdeveloped and developing countries is essentially nonexistent, primarily because milk production is insufficient even to supply fluid milk needs. Therefore, the possibility of manufacturing cheese in such countries is dependent mainly on the use of low-heat nonfat dry milk and vegetable fats and oils. The surplus of nonfat dry milk in western Europe and in the U.S.A. (Flanagan *et al.* 1978) and the abundant supply of vegetable fats and oils in the world at relatively low prices make these ingredients attractive

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[1]Paper No. 11988, Scientific Journal Series, Agricultural Experiment Station, University of Minnesota, St. Paul, Minnesota 55108

Journal of Food Processing and Preservation (1982) 15-30.

for use in cheese manufacture. The manufacture of good quality filled cheeses may help in supplying a high quality protein food for use in developing countries.

According to the literature the use of reconstituted nonfat dry milk with or without the addition of vegetable oils is less of a problem in the manufacture of soft than of hard cheeses. The manufacture of Cheddar cheese from reconstituted nonfat dry milk and vegetable fats has been studied (Peters 1956; Peters and Williams 1961; 1964; Rao-Jude and Rippen 1967; Rhodes 1966). Most of the filled cheeses manufactured were criticized as having flat, oily, beany, foreign or unnatural flavors and soft, weak, short, curdy, or crumbly body and texture characteristics. Some of these defects were detected also in Cheddar cheese made from whole milks containing large amounts of polyunsaturated fatty acids (Ahmed 1978; Czulak *et al.* 1974; Wong *et al.* 1972).

Although different types of plant lipids have been used, in most cases hydrogenation has been used to obtain the desired melting point. In developing countries, however, natural oils or fats having the desired melting properties are easier to obtain and use than are hydrogenated vegetable fats.

The purpose of this study was to investigate the possibility of manufacturing filled stirred curd cheese using commercial soybean and coconut oils and low heat nonfat dry milk reconstituted to different levels of total solids.

MATERIALS AND METHODS

Ingredients

Low-heat nonfat dry milk (NFDM) was obtained from Mid-America Dairymen, Inc. Soybean oil and coconut oil were purchased from Welch, Holme and Clark Co., Inc. (Harrison, NJ) unless it is indicated otherwise. Emulsifiers (mono- and diglycerides) were received from Continental Colloids, Inc.

Preparation of Filled Milks

Throughout the study, NFDM was reconstituted with the required amount of water, oil and emulsifier using the system shown schematically in Fig. 1. Filled milks were standardized to 3.5% (28% dry basis) fat and 12.5% TS or to 8.4% (28% dry basis) fat and 30% TS. The required amount of water was heated to about 38°C and poured into the blending vat; a mixture of the oil and 0.5% (oil base) mono- and diglycerides was then added. Next, the required amount of

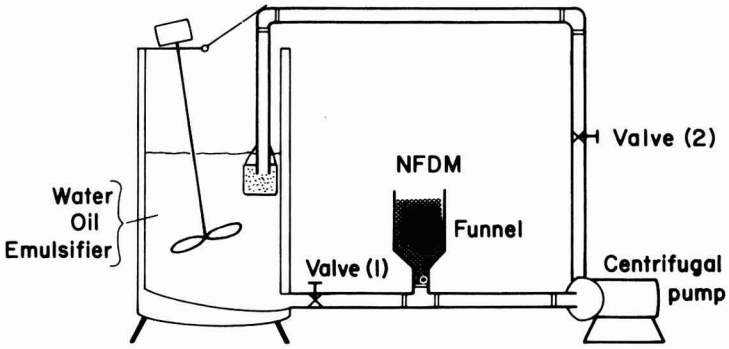

FIG. 1. THE SYSTEM USED FOR RECONSTITUTION OF NONFAT DRY MILK (NFDM) WITH WATER, OIL, AND EMULSIFIER TO THE DESIRED TOTAL SOLIDS CONTENT

NFDM was added through the funnel, from which it was circulated with the water and the oil through the centrifugal pump and back to the vat. The filled milks were then pasteurized at 63.8°C for 30 min, homogenized immediately at 500 psig, cooled and stored overnight at 1.7°C.

Cheesemaking

Soybean oil, coconut oil, and a 1:1 ratio of both were used to make filled milks for three replicate experiments wherein three vats of cheese were made: one for normal milk (control), the second for 12.5% TS reconstituted filled milk (R-12.5) and the third for 30% TS reconstituted filled milk (R-30). A minimum of 90 kg of milk was used in each vat. Where the 1:1 ratio of soybean oil to coconut oil was used, the soybean oil was a consumer packaged product (Hunt-Wesson Foods, Inc.). Sufficient dry calcium chloride to produce a concentration in the filled milks of 0.02% (W/W) was dissolved in 200 ml water and added just before renneting.

The stirred curd cheese-making procedure of Wilster (1951) was followed. When cheeses were manufactured from 30% TS filled milks, the procedure was modified based on extensive preliminary work (Mohamed 1980) which showed that acceptable cheese could be made from 30% TS concentrated milk by

1. Adding starter, color and salt at the normal rate (Wilster 1951) based on the amount of original milk (12.5% TS) used before concentration.
2. Adding rennet at the rate of 100 ml/454 kg of concentrated 30% TS milk.

3. Adding 31°C water, immeditely after cutting, at the rate of 30% of the amount of concentrated milk used.

The warm water facilitated stirring and handling of the curd and undoubtedly removed some of teh lactose and salts from the concentrated milk curd. Curd from each vat was hooped into 4.54 kg (10-lb) hoops, pressed overnight, removed from the hoops, coated with H. B. Fuller Adhesive (polyvinyl acetate co-polymer cheese coating) and cured at 4.5-7°C for at least one week and then waxed and put back at 7°C to ripen for at least three months. Control cheeses were made on one day and both filled cheeses were made the next day using the same bulk starter.

Chemical Analysis

A sample of milk from each vat was taken immediately before cheese making to determine TS, fat, total nitrogen and non-casein nitrogen. Whey samples were taken during draining to determine TS, fat and total nitrogen. Cheese samples were taken after one month to determine TS, fat, total nitrogen, salt and pH. The Mojonnier milk tester (Instruction Manual, 1925 Bull. No. 101) was used to determine total solids in milk and whey and fat in milk, whey and cheese. Total nitrogen was determined in milk and whey by Micro-Kjeldahl and in cheese by Macro-Kjeldahl (AOAC 1975). Salt in cheese was determined by the method of Wilster *et al.* (1937). Moisture content of cheese was determined using a moisture/volatiles tester Model SAS (C. W. Brabender Instrument, Inc.). Duplicate 10-g samples were heated at 130°C until constant weight was obtained (about 2 h). Acid development during cheese making was followed by determining pH of the whey using an Orion Research pH meter (Model 601A/digital Ionalyzer). The compositional data for milk, whey and cheese are presented on a dry basis (d.b.) for comparison purposes.

Sensory Evaluation

One- and three-month old cheeses were evaluated by a panel selected from among faculty and students in the Department of Food Science and Nutrition. An evaluation sheet similar to the American Dairy Science Association Cheddar Cheese score card was used. Judges were asked to score cheese flavor, body and texture and color on scales of 1-10, 1-5 and 1-5, respectively. They were asked to give an overall rating using the following terms: excellent, very good, good, fair, poor and very poor. They were also asked if they

would buy the lowest graded cheese. Cheeses were kept at room temperature for at least 1 hr before they were evaluated.

Objective Evaluation

General Foods Texture Profile Analysis as adapted to the Instron machine by Bourne (1968) was used to determine hardness, fracturability (brittleness) and springiness (elasticity). The Instron TM-M was equipped with compression cell CCTM (Bourne 1968, 1978; Lee *et al.* 1978) and operated at crosshead speed, chart speed and full scale settings of 2 cm min^{-1}, 10 cm min^{-1}, and 50 kg, respectively. At least 3 cylindrical cheese samples (27 mm diameter by 20 mm) were bored across the middle of each individual cheese which was cut horizontally. The samples were kept in airtight containers at 4°C until 1 h before the test when they were allowed to warm to room temperature. Each sample was compressed to 5mm (75% compression) in two consecutive cycles (two bites). The chart was set so that the second-bite curve appeared under the first. An example showing the determined texture parameters is provided in Fig. 2.

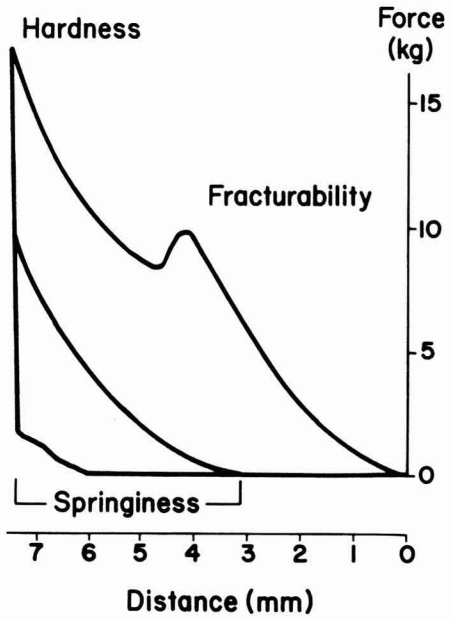

FIG. 1. FORCE-DISTANCE CURVE SHOWING SOME TEXTURAL PARAMETERS OF CHEESE

Statistical analysis of data by ANOVA (excluding fracturability) compared each type of filled cheese with the control and then compared each type of filled cheese with the others excluding the control. In all analyses the chosen probability level for significance of differences was 5%.

RESULTS AND DISCUSSION

Composition of Milks and Wheys

Total Solids. Composition of milks is shown in Table 1. Target compositions were not always achieved because of experimental errors and some losses during reconstitution.

During cheese making, the coagulum from 30% TS filled milk was definitely firmer and the curd was somewhat tougher than that from normal milk, whereas the coagulum from 12.5% TS filled milks was

Table 1. Composition of control and reconstituted filled milks and their wheys

	Oil Type	TS (Calculated)	TS	Fat (d.b.)	Protein (d.b.)
				%	
Milk	Soybean	Control	12.8	29.3	26.9
		12.5	12.4	26.7	25.5
		30.0	29.9	27.1	25.4
	Coconut	Control	12.7	29.8	25.0
		12.5	12.1	26.2	26.0
		30.0	30.3	28.3	25.2
	Coconut/ Soybean	Control	12.5	29.5	25.2
		12.5	12.5	26.5	25.5
		30.0	30.4	28.0	24.8
Whey	Soybean	Control	7.0	4.3	12.7
		12.5	7.0	2.1	12.2
		30.0	13.8	1.8	11.7
	Coconut	Control	6.9	4.4	13.1
		12.5	6.7	1.4	12.3
		30.0	13.7	1.9	11.8
	Coconut/ Soybean	Control	7.0	4.5	12.4
		12.5	6.9	1.5	12.3
		30.0	13.2	1.3	11.6

(d.b.) = dry basis
Results are means of three replicates

definitely softer than that from any of the other milks. There was no significant difference ($P < 0.05$) in TS content between whey obtained from 12.5% TS filled milks and that from normal milks (Table 1). As expected, TS was higher in wheys obtained from 30% TS filled milks. However, a lesser proportion of the original milk solids was lost in the whey from the higher (30%) total solids milks. This is probably because 30% TS filled milks produced firmer-bodied curd and fewer fine particles than 12.5% TS filled milks.

Fat. Fat content in the wheys from normal milk cheeses was significantly higher ($P < 0.05$) than that in wheys from filled milk cheeses, probably due to the homogenization effect (Peters 1956). There were no clear-cut differences in fat content of the wheys due to TS of filled milks.

Protein. Protein content of the wheys appeared not to be different.

Composition of Cheese

pH. Cheese made from 12.5% filled milks was higher in pH than normal milk cheese. The rates of acid production in the filled milk cheeses during manufacture were essentially the same (Fig. 3, 4 and 5). The higher pH in the 12.5% TS milk and curd than in the 30% TS

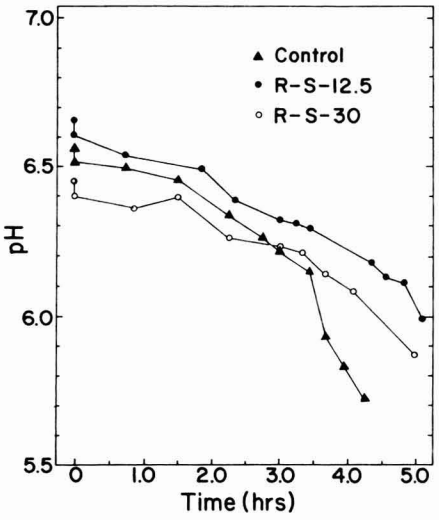

FIG. 3. pH VERSUS TIME DATA OBTAINED DURING CHEESE MAKING FROM NORMAL MILK (CONTROL) AND FROM NONFAT DRY MILK AND SOYBEAN OIL RECONSTITUTED TO 12.5% TS (R-S-12.5) AND 30% TS (R-S-30)

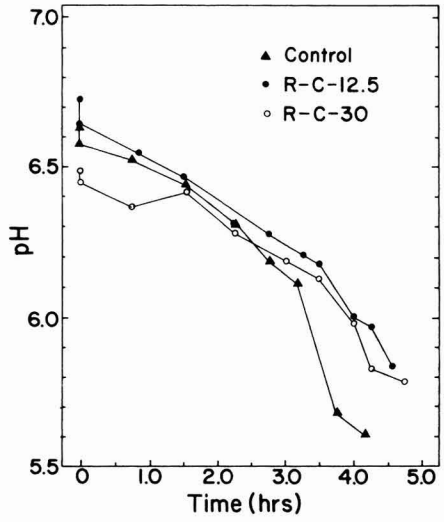

FIG. 4. pH VERSUS TIME DATA OBTAINED DURING CHEESE MAKING FROM NORMAL MILK (CONTROL) AND FROM NONFAT DRY MILK AND COCONUT OIL RECONSTITUTED TO 12.5% TS (R-C-12.5) AND 30% TS (R-C-30)

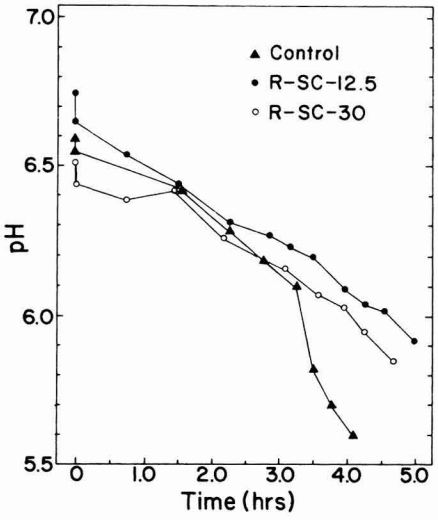

FIG. 5. pH VERSUS TIME DATA OBTAINED DURING CHEESE MAKING FROM NORMAL MILK (CONTROL) AND FROM NONFAT DRY AND A MIXTURE OF SOYBEAN AND COCONUT OIL RECONSITITUTED TO 12.5% TS (R-SC-12.5) AND 30% TS (R-SC-30)

milk and curd, initially and throughout the process, was due primarily to the lower solids concentration in the former. Both the rate and extent of acid development were greater in control curd than in filled milk curd toward the end of cheese making (between about 3 and 5 h). The rate of acid production was also greater in lots containing coconut oil than in those containing soybean oil (Fig. 3 versus Fig. 5). Lower acid production in the latter might have been due to the presence of free unsaturated fatty acids (Anders and Jago 1964; Czulak *et al.* 1974; Ahmed 1978) or fat oxidation products such as ketones and aldehydes.

Moisture. Moisture content of the curd was difficult to control during manufacturing of cheese from 12.5% TS filed milks. The moisture content of one-month old cheeses made from 12.5% TS filled milks was significantly higher ($P < 0.05$) than the moisture content in the control cheeses. However, moisture levels in 30% TS filled milk cheeses were not significantly lower than those of control cheeses (Table 2). This may have been due to the firmer coagulum and lower pH attained during cheese making from 30% TS versus 12.5% TS filled milk.

Table 2. Composition of one-month old stirred curd cheese made from fresh and from reconstituted filled milks

Oil Type	Milk TS %	pH	Moisture	Salt (d.b.)	Fat (d.b.)	Protein (d.b.)
			%			
	Control	5.2	40.0	2.9	51.5	39.6
Soybean	12.5	5.6	43.9	3.4	49.4	39.6
	30.0	5.4	38.3	2.4	49.7	37.9
	Control	5.2	40.31	2.8	52.6	39.1
Coconut	12.5	5.5	44.0	3.3	49.1	40.5
	30.0	5.4	39.3	2.4	49.7	37.7
	Control	5.3	40.83	3.0	52.7	39.2
Coconut/	12.5	5.5	42.3	2.9	50.7	40.0
Soybean	30.0	5.4	38.1	2.5	49.8	38.2

(d.b.) = dry basis
Results are means of three replicates

Salt. Salt content was significantly higher ($p < 0.05$) in cheeses made from 12.5% TS filled milks than in the other cheeses. Apparently, the higher the moisture content of the curd, the greater the rate of salt penetration into it, leading to a higher salt content in the cheese (Geurts *et al.* 1974).

Fat and Protein. Fat content of the control cheeses was somewhat higher than that of the filled cheeses. This is at odds with the data for fat losses in the whey (Table 1) and was probably due to a higher fat content in the control milk. Protein content of all cheeses was similar on a dry basis.

Yields

Cheese yields (Table 3) were almost the same from all milks and no significant differences ($p < 0.05$) were found among milks even when the yields were adjusted for constant moisture content (40%).

Table 3. Yield (lbs of cheese/100 lb of milk) for stirred curd cheeses made from fresh milk and reconstituted filled milks

Oil Type	Milk TS %	Yield	
		Actual	Adjusted to 40% moisture
Soybean	Control	10.5	10.5
	12.5	11.0	10.4
	30.0[a]	10.0	10.3
	30.0	23.5	24.5
Coconut	Control	10.9	10.8
	12.5	10.3	9.7
	30.0[a]	9.7	9.9
	30.0	23.9	24.7
Coconut/Soybean	Control	10.6	10.5
	12.5	10.3	9.8
	30.0[a]	10.1	10.3
	30.0	24.3	25.1

[a]Yield based on 12.5% solids milk
Results are means of three replicates

Sensory Evaluations

Flavor and Body and Texture. Numerical scores for flavor and for body and texture (Table 4) showed significantly higher quality in the control cheeses ($P < 0.05$) than in any of the filled cheeses. These results agreed with the findings of Peters (1956) and Rao-Jude and Rippen (1967). Filled cheeses made from 30% TS filled milks were sometimes graded higher ($P < 0.05$) in flavor as well as in body and texture than those made from 12.5% TS milks. Again, this may have been due to higher moisture content and slower growth of lactic acid bacteria in the 12.5% TS filled milk cheeses. The soybean oil filled

Table 4. Mean flavor and body and texture scores for one- and three-month old stirred curd cheeses made from fresh milk and reconstituted filled milks

Oil Type	% TS	Flavor		Body and Texture	
		1 mo.	3 mo.	1 mo.	3 mo.
Soybean[a]	Control	8.6	8.0	3.9	3.8
	12.5	6.6	6.2	3.0	2.7
	30.0	7.0	6.6	3.0	2.7
Soybean	Control	8.4	8.2	4.3	4.3
	12.5	4.5	5.3	2.6	2.3
	30.0	5.3	6.1	3.1	3.2
Coconut	Control	8.4	8.1	4.2	4.0
	12.5	6.8	6.8	3.1	3.0
	30.0	6.6	6.9	3.0	3.4
Coconut/Soybean	Control	8.6	8.2	4.3	3.8
	12.5	7.0	6.0	3.4	3.3
	30.0	6.4	6.6	3.4	3.3

[a]Soybean oil used was Hunt-Wesson consumer packaged product; values represent a single trial. Results for other oil types are means of three replicates

cheese made with Hunt and Wessons partially hydrogenated product scored higher in flavor than cheese made with the other soybean oil. A difference in the antioxidant content of the two oils may account for the differences in flavor scores. However, these results are not conclusive since only one trial was involved. Flavor scores and body and texture scores were about equal and inferior to the control among coconut oil and coconut/soybean oil filled cheeses. Soybean oil filled cheese was poorest in flavor and in body and texture. These sensory attributes appeared not to be influenced by total solids content of the reconstituted milk nor by age of the cheese.

Color. Although not all of the panelists scored cheese for color, the filled cheeses were lighter in color than the control, especially when soybean oil was used. The addition of annatto color before instead of after homogenizing did not intensify the color. A lighter color was also reported by Wong *et al.* (1972) in cheese made from normal milk that contained more than normal amounts of polyunsaturated fatty acids.

Overall Quality. Scoring for overall quality indicated that the control cheeses were good to very good, cheeses containing coconut oil or a mixture of coconut and soybean oil were poor to fair and those containing soybean oil were very poor to poor. When the judges were

asked whether or not they would buy the lower-scored cheeses, responses were about equally divided. In general, judges preferred filled cheeses made from 30% TS filled milks containing coconut oil or a mixture of coconut and soybean oil. When customers of the Department of Food Science and Nutrition sales store were asked to evaluate some of the filled milk cheeses, they were divided similarly to the panelists.

Flavor Criticisms. Oily, beany, unnatural and foreign flavors were noted by the panelists for the filled cheeses. Such flavors were also reported by Peters (1956) and Rao-Jude and Rippen (1967). The off-flavors were more pronounced in filled cheeses containing soybean oil and were more intense in the 12.5% TS versus the 30% TS product. Bitterness and oxidative rancidity were also more common in the products of lower total solids milks and less common in cheeses containing only coconut oil. The quality of oils used is obviously very important, and their degree of saturation is probably a factor in oxidative rancidity. Bitterness was detected in some of the normal milk cheeses. All of the filled cheeses were criticized as being flat and lacking typical cheese flavor. Those made from 30% TS filled milks were more flat than those made from 12.5% TS filled milks. Lack of typical cheese flavor in Cheddar cheese made from whole milk containing high levels of polyunsaturated fatty acids was also reported by Ahmed (1978), Czulak *et al.* (1974) and Wong *et al.* (1972).

The lack of flavor development in filled cheese appears to be due to slower growth of lactic culture organisms (Peters 1956) and resulting low concentrations of flavor compounds (Czulak *et al.* 1974). It could also be due to a smaller quantity of lower molecular weight fatty acids present in the vegetable oils compared to milk fat (Rao-Jude and Rippen 1967). The use of *Lactobacillus* cultures might have improved the flavor of the filled milk cheeses (Rhodes 1966; Abo-Einaga *et al.* 1974) particularly in cheeses made from milks containing greater than normal amounts of polyunsaturated fatty acids (Ahmed 1978; Czulak *et al.* 1974). The flavor of the filled cheese might also have been improved by controlling ripening conditions (Peters and Williams 1961; 1964) or by adding sweet cream buttermilk powder (Peters 1956).

Body and Texture Criticisms. The major body and texture criticisms were "dry," "crumbly," and "curdy." This was particularly true of the 30% TS filled milk cheese. Control cheese was the least curdy. During ripening, all cheeses become less crumbly and curdy.

Cheeses made from 12.5% TS filled milks were shorter in body than those made from 30% TS filled milks. Panelists criticized 12.5% TS filled milk cheese as being "soft," "weak," and "pasty" more often than they did the control cheese. The 30% TS filled milk cheeses were not soft, weak or pasty.

Objective Texture Measurement

The General Foods Texture Profile parameters of hardness, fracturability and springiness for one- and three-month old cheese are shown in Table 5.

Table 5. General Foods Texture Profile parameters for one- and three-month old stirred curd cheeses made from fresh milk and reconstituted filled milks

		Hardness (kg)		Fracturability (kg)		Springiness (cm)	
Oil Type	% TS	1 mo.	3 mo.	1 mo.	3 mo.	1 mo.	3 mo.
	Control	11.5	11.2	5.4	6.0	0.57	0.53
Soybean	12.5	9.6	7.0	3.1	2.6	0.59	0.58
	30.0	17.0	15.3	5.3	6.1	0.58	0.53
	Control	10.3	7.9	—	3.9	0.53	0.51
Coconut	12.5	18.0	12.8	0.0	0.0	0.74	0.79
	30.0	26.8	21.3	0.0	0.0	0.66	0.65
	Control	9.5	8.2	4.9	—	0.69	0.60
Coconut/	12.5	8.4	8.3	4.6	3.3	0.62	0.59
Soybean	30.0	14.3	14.2	8.9	6.0	0.70	0.62

Results are means of nine replicates

Hardness. Hardness values were significantly higher ($P < 0.05$) in filled than in control cheeses with the exception of the 12.5% TS soybean and coconut/soybean lots. Hardness values were considerably higher in coconut oil filled cheese than in the control; mean hardness of the lot made from 30% TS milk was nearly three times that of the control. Chen *et al.* (1979) also found that high-melting fats produced harder filled cheese than lower melting fats. Hardness was always higher in filed cheese made from 30% TS milk than in that made from 12.5% TS milk. This was probably due in part to the lower moisture content in the former (Table 2). Hardness decreased as the cheese was aged from 1 month to 3 months.

Fracturability. Fracturability was higher in filled cheese made from 30% TS milk than in that made from 12.5% TS milk. Coconut oil filled cheese did not exhibit fracturability. In some cases fractur-

ability peaks were not easily discernible as is shown in Fig. 6. Fracturability was most easily discerned in the soybean oil and soybean/coconut oil filled cheeses (Fig. 6). The information in Fig. 6 also shows the influence of moisture levels on shape and height of the compression curves.

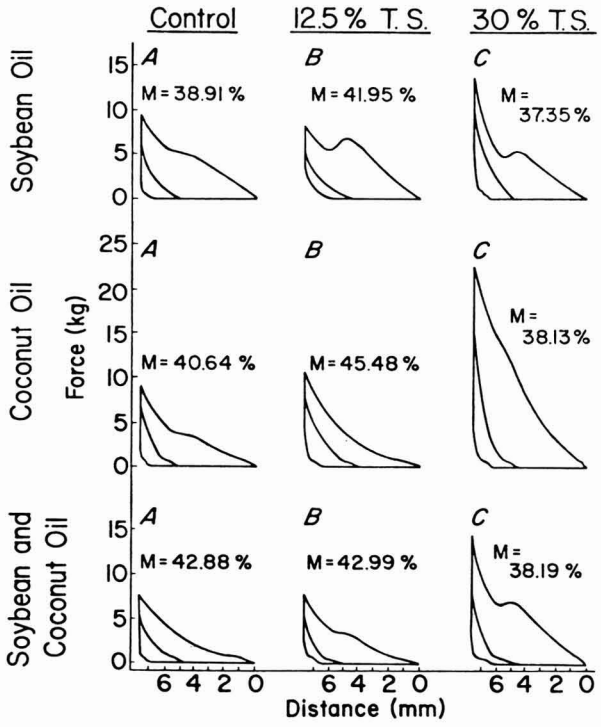

FIG. 6. TYPICAL EXAMPLES OF FORCE-DISTANCE CURVES OBTAINED BY INSTRON FOR THREE MONTH OLD CHEESES MADE FROM NORMAL MILK (CONTROL-A) AND FROM NONFAT DRY MILK WITH EITHER SOYBEAN OIL, COCONUT OIL OR A 1:1 RATIO OF BOTH RECONSTITUTED TO 12.5% TS (B) OR 30% TS (C) (M-MOISTURE)

Springiness. Springiness was highest in the 12.5% TS coconut oil filled cheese and generally higher in the coconut and coconut/soybean lots than in the soybean oil lots. No clear trend appeared as a result of aging.

CONCLUSIONS

The manufacture of stirred curd cheese from filled milks containing soybean oil, coconut oil, and a mixture of both was found to be feasible even though flavor and body and texture characteristics differed somewhat from those observed in normal milk cheese. Reconstitution of the dry milk to a higher than normal solids content improved the cheese making process and produced better body and texture in the cheese. The acceptance of filled cheeses by consumers in developing and underdeveloped countries must be determined; it may depend in part on the type of oil used. We recommend further studies using higher than normal levels of total solids and vegetable oil types selected to produce the desired melting point(s).

ACKNOWLEDGMENTS

The authors are grateful to Susan Halambeck for her assistance during this work.

This project was supported in part by project 18-79, University of Minnesota Agricultural Experiment Station.

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SURVIVAL OF VIRUS AFTER THERMOPROCESSING IN CAPILLARY TUBES

T. C. WHITE[1], N. D. HEIDELBAUGH[1] and S. McCONNELL[2]

[1]Department of Veterinary Public Health
[2]Department of Veterinary Microbiology and Parasitology
College of Veterinary Medicine
Texas A&M University
College Station, Texas 77843

Received for Publication December 1, 1981

ABSTRACT

A modification of the capillary tube heating technique described by Stern and Proctor was evaluated to determine its suitability for virus thermal inactivation studies. A suspension of Coxsackie virus B-5 (Faulkner Strain) in tissue culture maintenance medium was placed into melting point capillary tubes and heated to 56, 60, 72, and 80° C for various intervals of time. Surviving virus was quantitated by infection of cell cultures.

INTRODUCTION

A wide variety of viruses have been isolated from raw or partially cooked foods (Cliver 1976a; Cliver 1976b; Gerba and Goyal 1978; Larkin 1978; Martin 1974; Potter 1973). Occasionally, food products have been implicated in the transmission of viral diseases (Bryan 1972; Eyles *et al.* 1981; Leininger and Larkin 1980; Martin 1974). There is a need to increase our knowledge of the ability of viruses to survive in food. This need is particularly important in regard to foods rendered wholesome by limited heat treatment (Kophen and Potter 1981).

In technologically advanced societies, there has been a marked increase in the consumption of a wide variety of foods which are prepared and served outside of the home. Such practices afford the opportunity for exposure to many viral agents. Viruses may enter the food supply through contact with contaminated water or sewage, infected food handlers, rodent and insect vectors, and as a consequence of an infectious process in an animal from which food is derived (Becker 1966; Blackwell 1978; Larkin 1978; Larkin 1981; Lynt

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1966; Martin 1974; McKercher *et al.* 1978; Spencer *et al.* 1977). It has been suggested that the ingestion of viruses, particularly of animal origin, occurs frequently (Berg 1964).

Many viruses exhibit a narrow host range. This fact is attributed to specific receptor sites associated with the capsid proteins. Research has shown that loss of the capsid protein moiety of the virus can result in a broadening of the host range for some viruses (Larkin and Fassolitis 1979). The release of heat stable viral nucleic acid in the food supply as a result of heat treatment inadequate for inactivation could, therefore, initiate a novel health hazard. Recent innovations in heat processing, such as high-temperature-short-time (HTST) techniques, may not totally inactivate virus nucleic acid and may, in fact, contribute to its release from the intact virion (Larkin and Fassolitis 1979). In addition, the possibility of *in vivo* rescue of mildly heat damaged virions by cross reactivation or multiplicity reactivation has been suggested (Kophen and Potter 1981). This phenomenon has been demonstrated with partially denatured pox virus particles and vaccinia virus (Babbar and Chowdhury 1975; Joklik *et al.* 1960). Restoration of infectivity of thermally inactivated viruses would constitute a novel health hazard.

Viruses surviving in animal tissue can represent a significant threat to the health and production of livestock. Animal tissues distributed as food are reported as a possible vehicle for spread of economically devastating animal diseases such as Foot-and-Mouth Disease (FMD), Rinderpest, African Swine Fever (ASF), Swine vesicular disease, and Exotic Newcastle Disease (Becker 1966; Berg 1964; Blackwell 1976; Cottral *et al.* 1960; Hugh-Jones 1976; McKerscher *et al.* 1978).

Heidelbaugh and Graves were pioneers in systematic studies of food processing techniques on virus inactivation in animal tissues (Heidelbaugh and Graves 1968). Such products are now being processed by increasingly sophisticated techniques which require further evaluation regarding their influence on virus survival (Cliver *et al.* 1970; Cottral *et al.* 1960; Cunliffe and Blackwell 1977; Heidelbaugh and Graves 1968; Heidelbaugh and Giron 1969; Hyde *et al.* 1975; Kantor and Potter 1975; Kaplan and Melnick 1954a). The literature reports the survival of many viruses in foods and conflicting results concerning virus persistence after processing (Blackwell and Hyde 1976; Dimmock 1976; Flippi and Banwart 1974; Gough 1973; Hiatt 1964; Kaplan and Melnick 1952; Lynt 1966; Sellers 1969; Sullivan *et al.* 1967; Sullivan *et al.* 1971; Sullivan *et al.* 1975; Woese 1960). Such inconsistency is particularly evident in reports comparing the thermal resistance of FMD virus and human enteric viruses.

Viable FMD virus has been demonstrated in tissues heated to 80° C for four hours (Dimopoullos *et al.* 1959). In milk from cows infected with FMD, the virus was shown to survive ultra-high-temperature pasteurization (102° C to 138° C for 2-3 s). FMD virus was reliably inactivated only when the milk was heated to 148° C for three seconds (Cunliffe *et al.* 1979). Hepatitis virus and poliovirus has been shown to survive the pasteurization temperatures for milk, eggs, and mild cooking of meats (Becker 1966; Berg 1964; Dimmock 1976; Havens 1945; Kaplan and Melnick 1954a; Medearis *et al.* 1960; Strock and Potter 1972). Sullivan *et al.* reported that poliovirus which was readily inactivated in water heated to 71.1° C for 3 s survived a 15 s exposure to 79.5° C in cream and 25 s at 82.5° C in ice cream mix (Sullivan *et al.* 1975). Such data suggests that the medium in which the virus is processed may impart partial protection (Kaplan and Melnick 1954a).

Variations in thermal decay patterns of a virus may also be due in part to the test method used (Tierney and Larkin 1978). The predominant technique consists of mechanically adding the virus to a tube containing the food product either before or after the product is brought to processing temperature (Sullivan *et al.* 1971; Woese 1960).

The thermal lag involved in tube heating is minimized by the capillary tube heating technique described by Stern and Proctor (Farkas 1962; Stern and Proctor 1954). This procedure has been used in determining the termal stability of bacterial spores. However, few attempts have been made to evaluate its use as a virologic technique (Kaplan and Melnick 1954b; Licciardello and Nickerson 1962; Milo 1971).

This paper describes a modification of the capillary tube heating system for virus inactivation studies.

MATERIALS AND METHODS

Virus

Coxsackie virus B-5 (Faulker Strain) was employed in this study. Seed virus was obtained from the National Institute of Health. The derived virus pool was standardized as to titer, divided into multiple aliquots, and stored at −65° C until used.

Cell Culture

African green monkey kidney (Vero) cells obtained from the American Type Culture Collection (12301 Parklawn Drive, Rockville, MD 20852) were used to propagate and assay virus. Growth medium

consisted of Eagles Minimum Essential Medium (Earles Salts) supplemented with 2% fetal calf serum, 8% calf serum, 1% Non Essential Amino Acids, 1% Sodium Pyruvate, and antibiotics at a concentration of 100 μg/ml penicillin, 100 μg/ml streptomycin, and 100 μg/ml neomycin. Cell cultures were maintained with Basal Medium Eagles (Hanks Salts) supplemented with 2% horse serum, 2% Tryptose Phosphate Broth, and antibiotics as described above. Stock cells were propagated in 16 oz. flat sided prescription bottles and subcultured at regular intervals. For virus assay, confluent Vero monolayer cultures in 15 × 150 mm screw cap tubes were utilized. All cells were maintained at 35°C.

Virus Assay

Virus activity was determined by using serial ten-fold dilutions of sample. The sample (0.2 ml) was adsorbed onto confluent monolayers of Vero cells for one hour at 35°C. Maintenance medium was added to each tube and the cells were incubated at 35°C and observed for seven days. All samples were blind passed three times before samples were considered negative.

Heating of Samples

The effect of heat on survival of stock virus suspension was studied using a modified capillary tube technique. Stock virus suspension (0.02 ml) was added to each of 30 soda glass melting point capillary tubes (Kimax 51, 0.8-1.10 mm × 100 mm, Kimble Products Co.). Virus was added to each capillary tube with a Gilson Pipetman P20 micro-pipette (Rannin Instruments, Inc., Mack Road, Woburn, MA 01801) adapted with a 20 gauge two-inch hypodermic needle, or a Gilson Repetman R200 with a luer adapter and 20 gauge two-inch hypodermic needle.

The virus suspensions was deposited into the tube so that at least one centimeter of air separated the liquid phase from the opening of the tube, a precaution to prevent heating of the suspension during sealing. This distance was selected based on tests in which a thermocouple was placed at varying distances from the end of the tube during sealing. Temperature was determined with an Omega 0.020 inch Sub Miniature Type T (copper-constantan) therocouple and Model 405A Trendicator digital temperature readout (Omega Engineering, Inc., One Omega Drive, Box 4047, Stamford, CT 06907). Repeated trials showed that no rise in temperature occurred at 8-10 mm from the tube end during a five second application of a small flame at the tips. An oxygen-natural gas ampule sealer was pre-

ferred over a conventional gas burner. Both methods were tried and the ampule sealer was found to be superior since the sealing time was reduced to one or two seconds.

Five replicate tubes were used for each set of time temperature conditions (Table 1). This starting inoculum provided 0.1 ml of suspension, a convenient amount for the initial ten-fold dilution. Each group of five or more tubes was heated in a thermally controlled water bath maintained within 0.5°C of the desired temperature. Tubes were then chemically sterilized by immersion in chlorhexidine (Nolvason, Fort Dodge Laboratories, Fort Dodge, IA) for ten minutes

Table 1. Temperature and time intervals for heating virus suspension

Temperature	Time
56°C	10, 20, 30 and 60 min
60°C	0.5, 1, 5, 10, 15, and 20 min
72°C	2, 10, 20, 30, 60, 90, and 120 s
80°C	60 s

and then rinsed with 100 ml of sterile deionized water to remove residual disinfectant. Each group of tubes was placed in a sterile 10 × 100 mm test tube containing 0.9 ml of PBS diluent and crushed with a sterile 10 mm diameter glass stirring rod to release the content. Additional ten-fold dilutions through 10^{-8} were made and assayed for virus. Viral titers were determined by the method of Reed and Muench and the $TCID_{50}$ was calculated for each time and temperature. An additional group of five tubes containing diluent was assayed as a negative control.

EXPERIMENTAL RESULTS

A series of titrations were made using the stock suspension. Reproducibility was verified by 15 separate titrations with a mean titer of 8.1 logs $TCID_{50}$ ($10^{7.8} - 10^{8.7}$), and a standard deviation of 0.46.

The virus suspensions were then heated for the times indicated and results of subsequent assays are shown in Table 2.

Throughout the 72°C experiment, a significant delay in onset of first observable cytopathic effect was noted. This was in contrast to the unheated virus controls in which CPE was detected, even at

Table 2. Thermal inactivation of Coxsackie virus B-5

Temperature (C)	Time (min/*s)	Residual Virus (Logs)
56	0	8.1
	10	5.0
	20	3.2
	30	2.1
	60	2.2
60	0	8.1
	0.5	5.2
	1	2.3
	5	2.3
	10	1.6
	15	2.6
	20	1.7
72	0*	8.1
	2	2.3
	10	2.7
	20	1.4
	30	2.5
	60	3.2
	90	2.3
	120	2.7
80	60	1.2

*Time interval measured in seconds for 72° C and 80° C experiment

higher dilutions, within 24 h. However, in most cases this effect was delayed to approximately 72 h.

DISCUSSION AND CONCLUSIONS

Data obtained at temperatures of 56, 60, and 72° C was in agreement with inactivation curves previously described in the literature (Backrack *et al.* 1960; Dimmock 1976; Larkin and Fassolitis 1979; Milo 1971; Sullivan *et al.* 1971; Sullivan *et al.* 1975). These curves are biphasic, indicating the possibility of a two-step degradation process. The tailing effect occasionally reported in thermal inactivation studies may be due to either a stabilization of the virus by some component of the medium or the presence of a subpopulation of thermal resistant virions as previously suggested (Backrack *et al.* 1960; Larkin and Fassolitis 1979). Subsequent consecutive passage and heat treatments of progeny virus obtained from the 72° C-60

second trial failed to show an increase in titer as would be expected with a resistant subpopulation.

Members of the family *Picornaviridae*, notably the enteroviruses, are known for their persistance under adverse conditions, especially in the presence of stabilizing factors including divalent cations or proteinaceous material. The data presented here would suggest the protection or stabilization of the virus particle by one or more components of the suspending medium. Such a phenomenon could result from physical envelopment of the virion or aggregated virions or by stablization of chemical bonds which maintain the integrity of the capsid protein. Since RNA, the nucleic acid of the Picornaviridae, is itself resistant to thermal degradation at the temperatures used in this study, it is felt that any effect of heat on this component would be minor. The three-fold delay in onset of observable CPE at 72° C suggests damage to the virion, perhaps a subtle change in the spatial arrangement of the capsid protein (Dimmock 1976; Milo 1971). Such a change, while not completely denaturing the protein, could result in alteration of receptor sites with subsequent delay in normal attachment, penetration, and uncoating of the virus. Additional studies using more complex media are in progress.

ACKNOWLEDGMENTS

The authors wish to thank Prof. Daniel F. Farkas, Univ. of Delaware, for consultation and engineering design; and Prof. Walter Garcia Vidal, Universidad de la Republica, Uruguary, for consultation and encouragement.

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DEVELOPMENT AND STABILITY OF INTERMEDIATE MOISTURE CHEESE ANALOGS FROM ISOLATED SOYBEAN PROTEINS

M. MOTOKI[1], J. A. TORRES and M. KAREL[2]

Department of Nutrition and Food Science
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

[1]*Ajinomoto Co., Inc.*
Central Research Laboratories
1-1, Suzuki-Cho, Kawasaki-Ku
Kawasaki-City, Japan

Received for Publication January 10, 1982

ABSTRACT

Intermediate moisture cheese analogs (IMCA), based on soy beans, were fabricated by the blending method from an aqueous emulsion containing primarily soybean protein isolate, casein, hydrogenated vegetable oil, decaglycerol decaoleate, glycerol, salt and sorbitol. Potassium sorbate was used as a mycostatic agent.

Water activity of different IMCA preparations was controlled at levels of 0.83 to 0.91; and pH at 5.0, 5.5 and 6.5. Organoleptic properties, mechanical properties and microbial stability were tested. The IMCA product with a a_w of 0.87 and pH of 5.7 was the most acceptable, with a shelf life of about 3 months.

INTRODUCTION

Intermediate moisture foods (IMF) for human consumption have been available for centuries. In most of the IMF on the U.S. market, however, water activity (a_w) is depressed by either sugar or salt (Flink 1977). Recently, however, other humectants have been used to formulate IMF, either by blending or infusion (Heidelbaugh and

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[2]To whom correspondence should be addressed, at Room 16-311, Department of Nutrition and Food Science, M.I.T.

Journal of Food Processing and Preservation (1982) 41-53.

Karel 1975). Among products developed recently is an IMF cheese (Leung *et al.* 1976; Kreisman *et al.* 1978). Fermented soybean cheese has been developed in Japan (Matsuoka 1977; Fuji Oil 1977), but is subject to rapid deterioration.

The objective of this study was the development and evaluation of a more stable, IMF soybean-based cheese analog. The search for acceptable formulations was guided by 3 controlling parameters: texture as evaluated by compression tests, a_w and taste.

MATERIALS AND METHODS

Composition and Fabrication Procedure for IMCA

Several compositions were tried. The components are shown in Table 1. A brief description of IMCA fabrication is shown in Fig. 1. Glycerol (Certified A.C.S.; Fisher Scientific Co., Fair Lawn, NJ),

Table 1. Composition of the IMCA food system

	Sample No.								
	1	2	3	4	5	6	7	8	9
Ingredients	% w/w								
Isolated soybean protein	18.0	17.2	14.9	18.3	17.5	15.1	18.2	17.3	15.0
Na-Caseinate	4.1	3.9	3.4	4.1	3.9	3.4	4.1	3.9	3.4
Ca-Caseinate	1.3	1.3	1.1	1.3	1.3	1.1	1.3	1.3	1.1
Hydrogenated vegetable oil	22.4	21.3	18.5	22.7	21.5	18.7	22.6	21.4	18.6
Decaglycerol decaoleate	0.25	0.25	0.22	0.27	0.25	0.22	0.27	0.25	0.22
Salt	3.2	3.0	2.6	3.2	3.0	2.6	3.2	3.0	2.6
Glycerol	3.9	3.7	3.2	3.9	3.7	3.3	3.9	3.7	3.2
Sorbitol	12.7	12.1	10.5	13.0	12.2	10.6	12.8	12.2	10.6
K-Sorbate	0.25	0.25	0.22	0.27	0.25	0.22	0.27	0.25	0.22
Cheese flavor	0.55	0.60	0.54	0.67	0.61	0.52	0.62	0.61	0.56
Monosodium glutamate	0.8	0.09	0.11	0.12	0.13	0.10	0.12	0.11	0.11
Glucono-δ-lactone	—	—	—	0.17	0.16	0.14	0.72	0.68	0.59
Sodium aluminum phosphate	0.87	0.81	0.71	—	—	—	—	—	—
Water	32.4	35.5	44.0	32.0	35.5	44.0	31.9	35.3	43.8
a_w	0.85	0.87	0.91	0.85	0.87	0.91	0.85	0.87	0.91
pH	6.51	6.50	6.52	5.70	5.68	5.71	5.04	5.01	4.99
Moisture content (g H_2O/100 g solid)	50.2	57.6	81.4	49.2	57.5	81.3	49.1	57.1	81.0

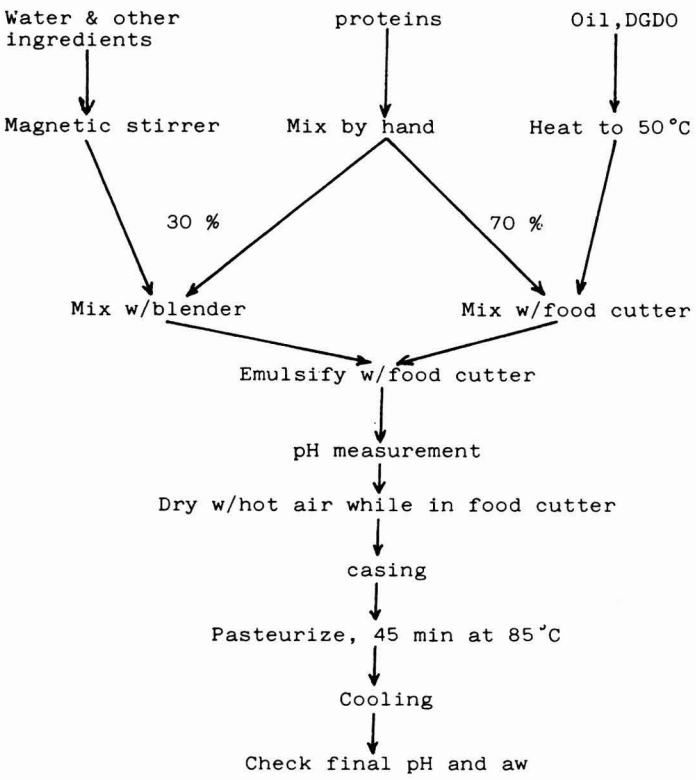

FIG. 1. FABRICATION OF INTERMEDIATE MOISTURE CHEESE ANALOGS (IMCA)

sorbitol (Pfizer Co., New York, NY) and sodium chloride (Baker Analytical Reagents, Phillipsburg, NJ) were dissolved in warm water (about 50° C) with potassium sorbate (Pfizer Co.) as the mycostatic agent, cheese flavor (Givaudan Co., Clifton, NJ) and monosodium glutamate (Ajinomoto USA Co., New York, NY) as the flavoring materials. The proteins used were: isolated soybean protein (Ajinomoto USA Co.) and caseinate (Erie Casein Co., Erie, IL). An emulsion paste was obtained by preblending 30% of the proteins in a Waring blender. The remainder of the proteins was mixed with oil phase composed of hydrogenated vegetable oil (Durkex 500; SCM Corp., New York, NY) and decaglycerol decaoleate (Glyco Chemical Inc., Greenwich, CT), and then emulsified using a food cutter (2 blades, 1450 rpm, Kitazawa Sangyo Co., Tokyo, Japan). This emulsion was then blended with the emulsion paste using the same food cutter.

After emulsification, the mixture was adjusted to the desired pH with glucono-δ-lactone (GDL) (FMC Co., Philadelphia, PA) or sodium aluminum phosphate (Stouffer Chemical Co., Westport, CT). Some of the water was then evaporated by blowing warm air (about 40° C) into the bowl of the food cutter until the desired a_w was reached. The emulsion was then filled into the seamless vinylidene chloride casing tube (diameter 20 mm, Kreha Chemical Co., Tokyo, Japan) with a hand press.

The emulsion was pasteurized at 85° C for 45 min. After cooling, pH was determined with a surface electrode probe (Beckman Model 39507) and a_w with an electric hygrometer (Sina-Beckman, Cedar Grove, NJ).

Moisture Content Determinations

The moisture content of all samples was measured by the vacuum oven method as follows: duplicate samples were accurately weighed into weighing dishes equipped with tight-fitting covers. The IMCA samples in the weighing dishes, with loosened covers, were placed in a vacuum oven at 29 in. of Hg and 60° C for 25 h. This relatively low-drying temperature was selected to minimize humectant losses. At the end of the drying period, the vacuum was broken with air flowing through a bed of $CaSO_4$ (Drierite; W. A. Hammond Drierite Co., Xenia, OH), the covers closed and samples weighed as soon as they reached room temperature. The weight change was recorded as moisture content.

a_w Determinations

Each sample, in duplicate, was equilibrated to 30° C ± 0.5° C for 2 h before measuring its a_w with an electric hygrometer. Calibration of a_w was carried out using the values of the saturated salt solution of sodium chloride, potassium chloride, ammonium sulfate, potassium nitrite and potassium sulfate reported by Wexler and Hasegawa (1954).

Microbiological Challenge Test

Each of the IMCA was challenged with 3 different microorganisms: 2 molds (*Aspergillus niger* and *Aspergillus tonophilus* and a bacterium (*Staphylococcus aureus* S-6). Procedures for the microbial testing have been summarized in Fig. 2. Initial inoculation level and change in number of *S. aureus* strain S-6 was determined by plating on brain-heart-infusion (BHI; Difco, Detroit, MI)

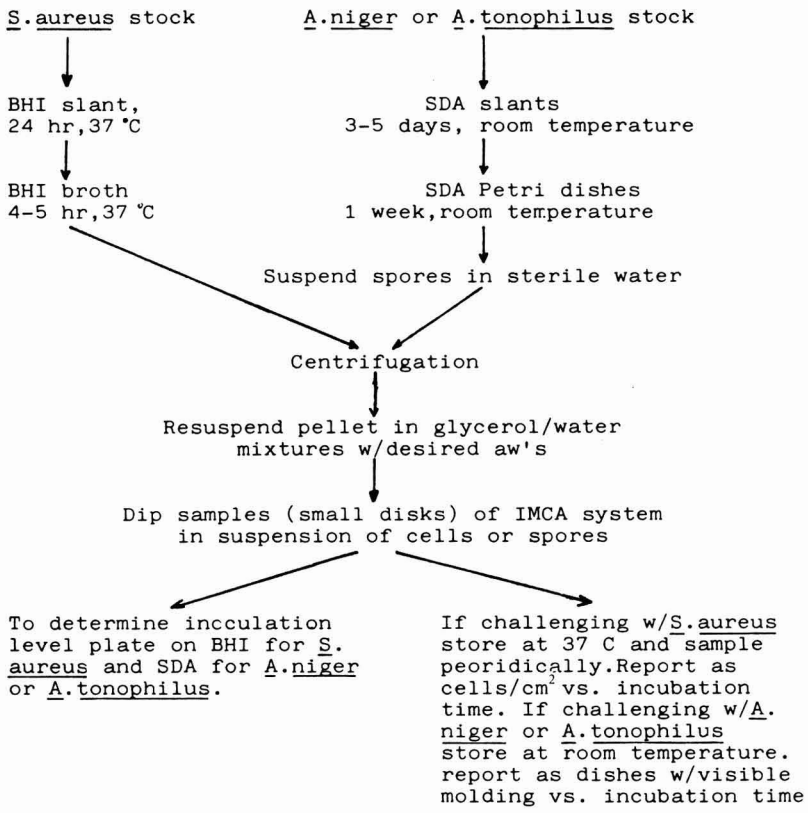

FIG. 2. INOCULATION PROCEDURE FOR IMCA FOOD SAMPLES

agar plates. The inoculum of *A. niger* and *A. tonophilus* was obtained by plating on Sabourad dextrose agar (SDA; Difco). Occurrence of outgrowth of these molds was determined by direct visual examination of inoculated samples. The samples were placed in sterile petri plates and stored in a dessicator at an a_w adjusted with sterile glycerol-water mixture to that of the specific sample.

Texture Measurement

IMCA samples were compressed in an Instron Universal Testing Machine, model 1122 (Instron Corp., Canton, MA). The conditions of measurement are 20 mm/min in crosshead speed, 50 mm/min in chart speed and 60% of deformation ratio with load cell (Model 2511-302). Analysis was carried out by Bourne's method (Bourne 1968).

Parameters measured were: compression force required for the given deformation, or "hardness"; the force at the elastic limit, often called "breaking force"; the work ratio of the first two cohesive compressions, or "cohesiveness"; the adhesive force exerted in the ascending motion of the compressing plate after the first bite; and the elastic recovery (or the recovered height in mm after the first bite), corresponding to "springiness". All experiments were replicated at least 3 times. Average values are reported.

Organoleptic Tests

IMCA samples were evaluated by a 15-member taste panel comprised of both male and female members of the Department of Nutrition and Food Science on a 9-point hedonic scale (9 = like extremely; 5 = neither like nor dislike; 1 = dislike extremely).

RESULTS AND DISCUSSION

Formulation of IMCA

Table 1 shows a_w, pH and moisture content of each formulation. The formulations were chosen on the basis of preliminary trials. Some of the formulation variations which were tried are discussed below: pH and a_w were varied to test the effects of the above factors on fabrication and microbial stabilities. Small amounts of caseinate were added to obtain the cheeselike texture, and hydrogenated vegetable oil was used to increase emulsion stability during storage at room temperature. Sorbitol was substituted for sugar to suppress sweetness and minimize browning. Reduction of final moisture content was achieved by drying in warm air, because the moisture content required for the emulsification step was higher than the level desired for storage stability.

Evaluation of Texture

Results of texture measurements of IMCA foods before storage are shown in Table 2. Apparent compression force or "hardness" increased steadily with decreased initial a_w of the IMCA, but work ratio, or "cohesiveness" did not increase as much. Work ratio decreased and the texture tended to become weak and brittle with decreased pH. Generally, IMCA was tougher than some commercial cheeses, but 2 IMCA formulations (Table 1: #5 and #8) had texture patterns similar to commercial cheeses.

Table 2. Texture measurement of IMCA before storage[a]

Material	Type	Apparent Compression Force[b] (g/cm^2)	Work Ratio (%)	Recovered Height (mm)	Adhesive Force (g/cm^2)	Force at Inflection Point (g/cm^2)
IMCA	1	1526	36	5.0	—	—
	2	1180	39	5.2	—	—
	3	510	41	3.6	—	—
	4	1310	22	2.1	—	340
	5	873	24	2.0	18	490
	6	490	30	1.4	60	—
	7	1150	19	2.4	—	510
	8	1040	18	1.6	53	390
	9	350	20	2.0	33	—
Cheese	American	560	11	0.6	55	135
	Swiss	552	14	1.1	—	190
	Mild cheddar	467	17	0.8	55	62

[a]Instron Universal Testing Instrument, model 1122. Conditions of measurements are as follows: 0%, deformation ratio; 20 mm/min, crosshead speed; 10 mm, sample height; model 2511-302, load cell
[b]Apparent values because of increase in area of the sample cross-section during the compression

Table 3 shows changes of compression force and work ratio during storage of IMCA at room temperature and with humidity in equilibrium with the a_w of the given IMCA. The data show a significant increase in toughness during storage for IMCA with pH's of 6.5 and 5.7. The low pH samples tended to deteriorate due to emulsion breakdown.

Microbial Growth

S. aureus: A challenge test was conducted at 3 a_w's and 3 pH's. Results are shown in Fig. 3-5. The growth curves in these 3 figures led to the following conclusions:

1) Even at the lowest a_w tested (about 0.85), increase in cell number was detected, but this increase (about one log cycle in some of the extreme conditions) may represent recoveries rather than growth.

2) Stability was greatly increased by decreased pH. At pH 5, a_w 0.91, no increase in colonies was detected for 7 days (Fig. 3). At the lowest a_w, cell numbers decreased (Fig. 3-5). These results are somewhat inconsistent with the results at the intermediate a_w (0.87), where growth was detected at the beginning of the test, but subsequently cell numbers decreased (Fig. 3-5). pH may have an effect by

Table 3. Measurements of compression force and work ratio of IMCA during storage at room temperature

IMCA Formulation #		DAYS 1	7	21	45	66	90	125	150
1	C.F.	1526	1650	1820	2090	2120	2350	2500	2670
	W.R.	36	36	39	42	42	44	44	47
2	C.F.	1180	1250	1400	1450	1620	1750	1900	2050
	W.R.	39	40	41	43	44	45	46	47
3	C.F.	510	525	537	565	586	605	619	624
	W.R.	41	41	41	42	42	43	44	44
4	C.F.	1310	1370	1450	1700	1895	2150	2350	2510
	W.R.	22	23	26	29	31	34	36	38
5	C.F.	873	895	950	1100	1220	1245	1200	1220
	W.R.	24	25	27	29	32	32	32	33
6	C.F.	490	510	525	545	560	572	586	589
	W.R.	30	31	31	32	33	33	34	34
7	C.F.	1150	1140	1120	1000	998	[a]	[a]	[a]
	W.R.	19	18	18	15	15	—	—	—
8	C.F.	1040	1060	1045	1057	1100	1125	[a]	[a]
	W.R.	18	18	18	18	19	20	—	—
9	C.F.	350	357	345	341	345	335	325	[a]
	W.R.	20	20	20	19	19	17	16	—

C.F. = Apparent compression force (g/cm^2); W.R. = Work ratio (%)
[a]Oil separation observed

itself and also by promoting the effectiveness of the preservative used in this study.

3) Decrease-increase (in cell numbers) cycling was detected in some experiments, which may be due to one or a combination of the following reasons: (a) during growth, cells exhaust easily available nutrients and further growth requires diffusion of nutrients; an inhibitory compound is excreted to the environment and further growth requires diffusion of nutrients; (b) an inhibitory compound is excreted to the environment and further growth requires dilution by diffusion; (c) cells may grow and then die, releasing nutrients that promote new growth; or (d) a acidic metabolic products are excreted, pH surrounding the cells decreases and the effectiveness of the preservative increases. A determination of the precise cause of the phenomena would require analysis beyond the scope of this stability test.

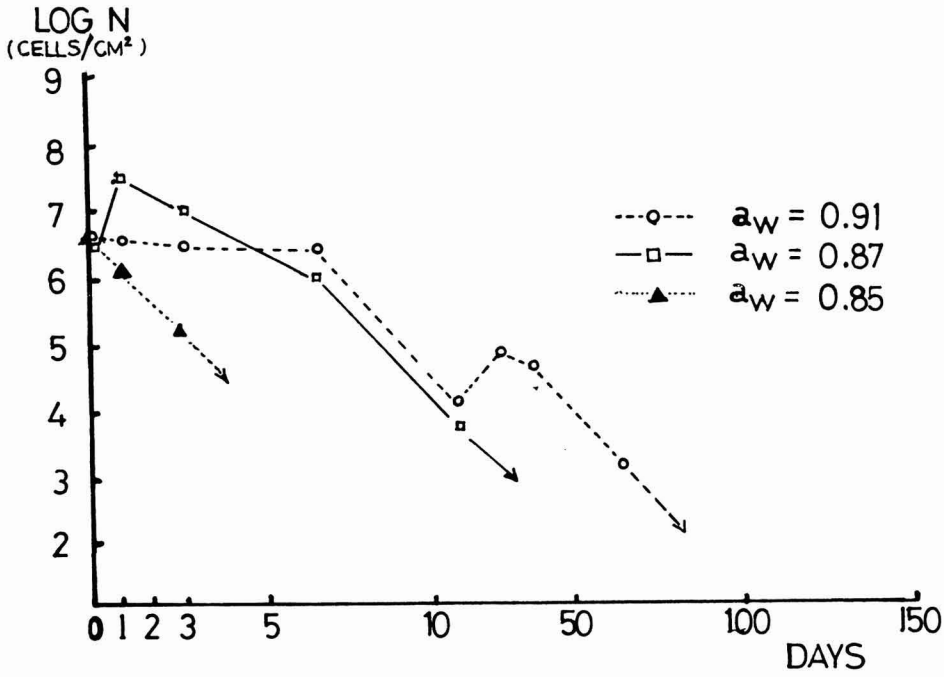

FIG. 3. *S. AUREUS* GROWTH AT pH 6.5 AND VARIOUS a_w LEVELS

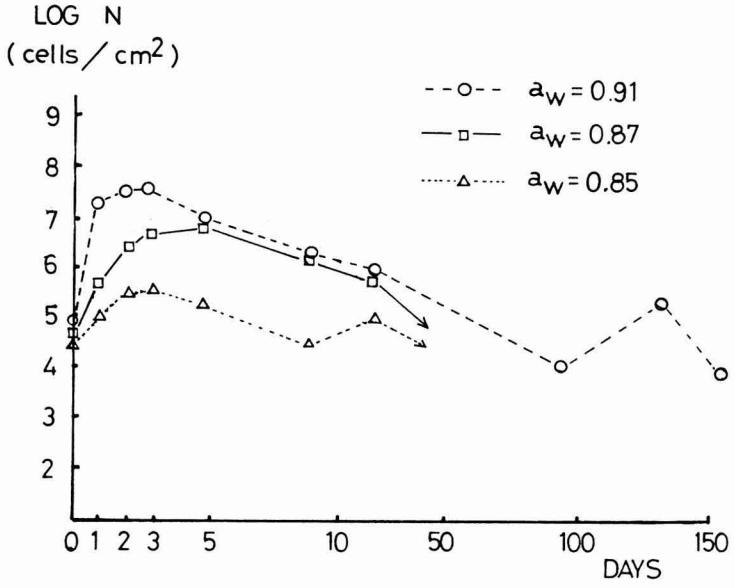

FIG. 4. *S. AUREUS* GROWTH AT pH 5.5 AND VARIOUS a_w LEVELS

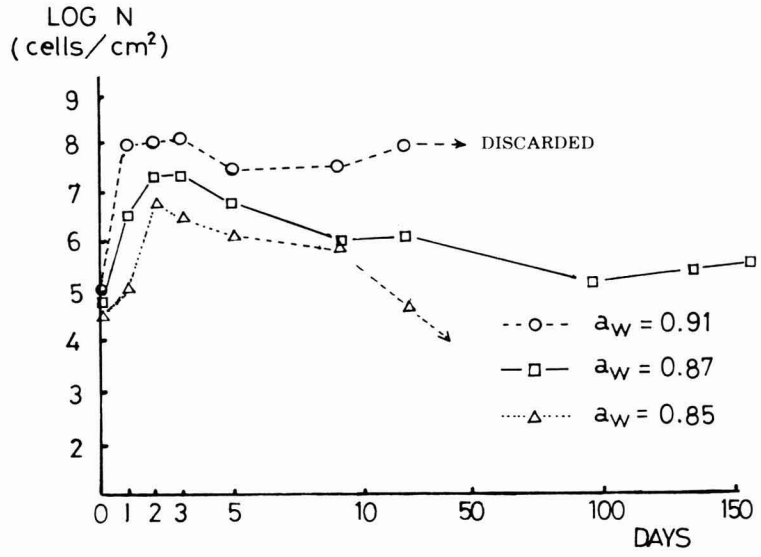

FIG. 5. *S. AUREUS* GROWTH AT pH 5.0 AND VARIOUS a_w LEVELS

In general, the challenge experiments using *S. aureus* S-6 showed that effect of pH on stablity is highly significant. In practice, however, substantial pH decrease is not possible, due to loss of acceptable texture and taste.

A. niger and A. tonophilus: The procedures for challenge tests are shown in Fig. 2. The inoculum levels were 30 to 70 spores per cm^2 for *A. niger* and 20 to 30 for *A. tonophilus*. Results of the challenge are summarized in Tables 4 and 5, which indicate the number of petri dishes that showed visible molding. The total number of petri dishes used was 10; 1 for initial inoculum determination and the other 9 for incubation at room temperature under controlled humidity conditions.

It was found that pH contributes significantly to the stability of the IMF tested. As the pH of the samples was decreased, the time required for detection of molding increased. *A. niger* seems to be a more serious challenger than *A. tonophilus*.

Table 4. *Aspergillus niger* challenge studies: number of samples out of 9 showing visible molding

pH	a_w	Days													
		2	4	5	9	12	15	18	25	40	57	76	97	174	220
5.5	0.90				9										
	0.87				1	3	9								
	0.85									3	3	4	4	5	5
	0.83														...›
6.0	0.87			7	9										
	0.85				9										
	0.83						1	8	9						
6.5	0.85			6	9										
	0.83					9									

Table 5. *Aspergillus tonophilus* challenge studies: number of samples out of 9 showing visible molding

pH	a_w	Days													
		2	4	5	9	12	15	18	25	40	57	76	97	174	220
5.5	0.90						5	9							
	0.87									1	1	2	2	2	2
	0.85														...›
	0.83														...›
6.0	0.87							4	9						
	0.85									4	5	5	5	5	5
	0.83														...›
6.5	0.85					3	7	9							
	0.83					9									

Organoleptic Evaluations

The results of the taste panel ratings before storage are shown in Table 6. Acceptability increases with increasing a_w and pH. Table 7 shows number of days to unacceptability (a rating of 5 = neither like nor dislike). Formulation #3 was most stable with over 15 months organoleptic shelf life. However, it is the most susceptible to microbial growth, including growth of *S. aureus*. The most acceptable formulation is #5, with about 3 months shelf life.

Table 6. Organoleptic tests on IMCA before storage

IMCA Formulation	Taste Scores	Texture Scores	Overall	Comments
1	4.93	5.92	5.25	too tough
2	5.00	6.34	5.54	
3	6.56	6.88	6.79	good
4	4.76	4.73	4.65	very dry
5	5.05	6.01	5.78	
6	5.27	6.09	5.97	
7	4.40	4.63	4.53	very brittle
8	4.89	5.05	4.79	crumbly
9	5.72	5.25	5.31	

The difference between samples were not significant at the 5% level.
Scale: 9 = like extremely; 5 = neither like nor dislike; 1 = dislike extremely

Table 7. Acceptability of stored IMCA samples

Sample	Days to Organoleptic Unacceptability
1	21
2	35
3	455
4	0
5	9
6	350
7	0
8	0
9	35

ACKNOWLEDGMENTS

The authors thank Professor Chokyun Rha for her advice and assistance in the textural aspects of this study and Professor Reinaldo Gomez for his advice and assistance in the microbiological aspects of this study. The authors gratefully acknowledge also the financial support of Ajinomoto Co., Inc. and the fellowship granted by Westreco to one of the authors (J.A.T.).

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