further heated before consumption, upon which the amount of residues again decreases.

The observations concerning MFH residues made in this work agree with those of other researchers and confirm that false morel mushrooms should be processed with great care. The method described for the determination of MFH compounds, although precise and suitable for control procedures, requires high gas chromatographic separation efficiency and careful selection of the correct column. The FFAP glass capillary column should be neutral or slightly basic since MFH compounds are not quantitatively determined in acid conditions, leading to the possibility of faulty determinations. However, standards for comparison can be easily prepared from commercial chemicals, although because of their tendency to decompose, the purity of MFH compounds should be repeatedly confirmed, for example, using ^{1}H NMR.

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Received for review September 20, 1976. Accepted December 27, 1976. This work was supported by the Foundation for Research of Natural Resources in Finland (Suomen Luonnonvarain Tutkimussäätiö).

Physical Stability of Milk Fat Emulsions after Processing as Evaluated by Response Surface Methodology

Lloyd M. Smith,* Michael B. Carter, Toshiko Dairiki, Armando Acuna-Bonilla,[1] and William A. Williams

Computer analysis of a response surface experimental design was used to evaluate the effect of three variables, homogenization temperature (50, 60, 70 °C), pressure (1000, 1500, 2000 psi), and emulsifier concentration upon physical stability of 25% milk fat emulsions. The first series of emulsions contained nonfat milk solids (NFMS) at 1, 6, and 11% levels, but no commercial emulsifier. The second and third series contained 1% sodium caseinate and 9% NFMS, respectively, plus emulsifier. Emulsifiers and their concentrations in the fat phase were: RG Lecithin at 0, 0.5, and 1.0%; Emplex at 0, 0.05, and 0.1%; and Tween 20 at 0, 0.5, and 1.0%. A small-scale pilot plant homogenizer was used to prepare each emulsion. Stability data for each experiment were analyzed by fitting a Taylor second-order equation for three independent variables. Response surface plots were generated by the computer for emulsion stability as a function of temperature vs. pressure at the selected NFMS or emulsifier level. In each plot there were many combinations of temperature and pressure that would produce the same response. Pressure generally was more important than temperature or emulsifier level in determining stability. However, a variety of curvilinear interrelationships among the variables was evident from the different forms of the curves (rising or falling ridges, bull's-eyes, saddles). A combination of variables can be selected by this procedure to give an optimum or a desired emulsion stability.

Many formulated foods are made by emulsifying liquid fat into an aqueous phase containing proteins, carbohydrates, and other materials. The nutritional and functional properties of such food emulsions are of great importance to the processor and consumer. There is a need to develop improved laboratory and pilot plant procedures to guide the formulation and preparation of new foods and to improve current processing practices. In our previous research (Smith and Dairiki, 1975a,b), techniques were reported to prepare and evaluate the physical stability of model oil-in-water emulsions as influenced by sodium caseinate, nonfat milk solids (NFMS), and different emulsifiers. With more complicated systems, interference occurred between some emulsifiers and the other components in experiments where we varied one ingredient at a time while other components were held "constant". It became apparent that emulsion variables cannot be assumed to act independently as implied by single-factor experiments.

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Table I. Coding of Independent Variables for RSM Study of Stability of Model Emulsions Containing 25% Milk Fat

Variable	Code	−1	0	+1
Homogenization pressure, psi	X_1	1000	1500	2000
Homogenization temperature, °C	X_2	50	60	70
NFMS, % in aqueous phase	X_3	1	6	11
Emulsifier, % in fat				
RG Lecithin	X_3	0	0.5	1.0
Emplex	X_3	0	0.05	0.1
Tween 20	X_3	0	0.5	1.0

Table II. Stability Indices[a] for Emulsions Prepared at 60 °C by Homogenization at Different Pressures

Replications	Pressure, psi 500	1000	1500	2000	2500
Day 1	25.9	45.2	65.3	66.6	72.8
Day 2	26.5	41.8	58.1	64.6	69.0
Day 3	26.9	49.0	58.6	66.0	69.4
Mean[b]	26.4v	45.3w	60.7x	65.7y	70.4z
SD	0.34	2.3	3.4	1.4	1.6
CV	1.3	5.0	5.6	2.2	2.3

[a] Averages of four replicates. [b] Means followed by the same letter do not differ significantly at $p < 0.05$ (*FLSD*, Carmer and Swanson, 1971), i.e., each mean differs from all others.

Table III. Effect of Rapid Cooling to 3 °C on Stability Indices[a] of Milk Fat–NFMS Emulsions Prepared at 60 °C by Homogenization at Different Pressures

	Pressure, psi 500	1000	1500	2000	2500	Mean[b]
Control	27.8	52.9	66.4	71.4	73.2	58.3u
Cooled	32.6	54.4	70.0	74.8	76.2	61.6v
Mean[b]	30.2w	53.6x	68.2y	73.2z	74.7z	

[a] Average data from eight Stability Index determinations. [b] Means followed by the same letter do not differ significantly at $p < 0.05$ (*FLSD*, Carmer and Swanson, 1971).

Becker (1967) introduced the use of response surface equations and graphs to illustrate the effect of several preparation parameters on the initial size distribution in oil-in-water emulsions. Hill and Hunter (1966) reviewed the literature of response surface methodology (RSM), emphasizing the fields of chemistry and chemical engineering. Myers (1971) has written a mathematically oriented textbook on RSM. In 1972, Henika proposed a simple RSM procedure that revealed interactions between variables and illustrated its potential application in the food industry. Since then, RSM has been employed in food science including studies of bacterial growth (Schroder and Busta, 1973), protein denaturation (Nielsen et al., 1973), high protein bread (Henselman et al., 1974), and soybean extruded product (Aguilera and Kosikowski, 1976). Thus, multiple factor experiments are necessary to test the effects of emulsion variables on the stability of the emulsion.

In the present study we used RSM as a tool to show the effect of three variables, homogenization temperature, homogenization pressure, and emulsifier concentration, on the stability of three series of emulsions containing different levels of sodium caseinate or NFMS in the aqueous phase. An improved computer-controlled procedure was developed to produce hard-copy graphs illustrating the results (see the Appendix). We conclude that RSM has great potential in evaluating the composite influence of the numerous variables that affect the properties of food emulsions. The approach employed in this study should be valuable in related fields.

Table IV. Treatment Combinations and Stability Indices for Emulsions Containing Three Levels of NFMS

Trial[a]	Variable P, psi X_1	T, °C X_2	NFMS, % X_3	Stability index
1	1000	50	6	46.7
2	2000	50	6	14.6
3	1000	70	6	26.8
4	2000	70	6	41.1
5	1000	60	1	3.7
6	2000	60	1	4.1
7	1000	60	11	41.7
8	2000	60	11	66.6
9	1500	50	1	4.1
10	1500	70	1	3.8
11	1500	50	11	51.9
12	1500	70	11	78.5
13	1500	60	6	34.6
14	1500	60	6	30.5
15	1500	60	6	32.1

[a] Trials were run in random order.

EXPERIMENTAL PROCEDURE

Apparatus. The equipment used for sample homogenization consisted of (a) a high-pressure, controlled-volume Milton Roy pump; (b) an Ormerod homogenizing valve with pressure manometer; and (c) a parallel-flow heat exchanger placed between the pump and the homogenization valve. Preliminary experiments established equipment residence volume, flow rates, and heat transfer conditions for processing the emulsions at the desired temperatures and pressures.

Determinations of total solids were made gravimetrically with a Mojonnier total solids tester (Milk Industry Foundation, 1959).

Materials. A single lot of anhydrous milk fat for emulsion preparation was supplied by Foremost Food Co., San Francisco, Calif. Aliquots of the melted fat were washed with distilled water and centrifuged. The fat contained no phospholipids and its fatty acid composition was within the normal range. Wiley melting point was 34 °C, refractive index at 40 °C was 1.4541, and titratable acidity as lactic acid was 0.01%.

The emulsifiers used were Emplex (sodium stearoyl-2-lactylate, Patco Products Co., Kansas City, Mo.), RG Lecithin (dietary soybean phospholipids, Central Soya, Chicago, Ill.), and Tween 20 (polyoxyethylene sorbitan monolaurate, Sigma Chemical Co., St. Louis, Mo.).

NFMS was Sanalac instant nonfat dry milk produced by Beatrice Foods Co., Madison, Wis. Edible sodium caseinate was Savortone LF from Western Dairy Products, San Francisco, Calif.

Preparation of Emulsions. The aqueous phase of the emulsions was prepared by dispersing the specified amount of NFMS or sodium caseinate in deionized distilled water and allowing the solutions to hydrate overnight at 5 °C.

All emulsions were 500 g in weight and contained 25% by weight of milk fat. The required amount of emulsifier was dispersed in the liquid fat which was weighed into the aqueous phase and held at 52 °C for 15 min. Each mix was blended for 10 s in a Waring blender at low speed and then immediately passed through the homogenization apparatus. Before each mix was processed, the required homogenization temperature was adjusted with the parallel flow heat exchanger, using water previously warmed to 50 °C in the supply funnel. The first 100-mL portion of each

Table V. Analysis of Variance for Emulsions Containing Three Levels of NFMS

Source	df	SS	Mean square	F
Due to regression	9	7297.9		
Linear				
X_1	1		7.0	0.1
X_2	1		135.6	1.9
X_3	1		6219.5	89.1[a]
Quadratic				
X_1^2	1		33.4	0.5
X_2^2	1		28.5	0.4
X_3^2	1		1.1	0.0
Interaction				
X_1X_2	1		541.0	7.8[b]
X_1X_3	1		150.2	2.2
X_2X_3	1		181.8	2.6
Deviation from regression	5	348.9	69.8	
Total	14	7646.8		
Coeff of determination R^2	0.95			

[a] $p < 0.001$. [b] $p < 0.1$.

emulsion was discarded during sample collection.

Determination of Emulsion Stability. Because the emulsions produced were relatively stable to gravity, the stability index method reported previously (Smith and Dairiki, 1975a) was modified by centrifuging the samples on the day of preparation. Two samples of each emulsion in 12-mL graduated tubes were centrifuged at 3000 rpm for 15 min at 24 °C in a clinical centrifuge (International, Model CL). The top half of the sample was aspirated off and the percent total solids in the bottom half was determined in duplicate. The "stability index" was obtained by dividing the average total solids percentage in the bottom half by the calculated total solids of the original sample, and multiplying the result by 100. Three or four test emulsions were prepared and their stability indices determined in 1 day.

RSM Experimental Design. The fractional factorial design chosen was the three-variable, three-level central composite design described by Henika (1972). Variable levels were coded as −1, 0, +1 (Table I), and the order of performing the tests was selected randomly. Lower and upper variable limits were selected by considering previous emulsion stability experiments and practical composition and processing conditions. Stability index data were analyzed for multiple regression using a Burroughs 6700 computer and a standard statistical computer package (Dixon, 1970).

Regression equations were obtained by fitting a Taylor second-order model in three variables:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{33}X_3^2 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3$$

where Y is the response (stability index), β_0 is a constant, β_1 is the regression coefficient for X_1, β_{11} is the coefficient for the quadratic term of X_1^2, and β_{12} is the coefficient for the X_1X_2 interaction, etc.

The ten regression coefficients were fed into a Tektronics 4010 terminal to develop graphical plots on the display screen, using a computer program described in the Appendix. After a satisfactory plot with appropriate contour intervals was obtained, the information was transmitted to a Calcomp Plotter to produce hard-copy graphs suitable for photographic reproduction and publication.

RESULTS AND DISCUSSION

Variability in Emulsion Preparation and Stability Evaluation. Before the influence of processing variables could be evaluated, it was necessary to obtain data on flow rate, residence volume, and heat transfer capacity of the homogenizer system. This information was used as a guide in preparing model emulsions whose stability indices were determined. Representative results for emulsions containing 9% NFMS in the aqueous phase and prepared with different homogenization pressures on 3 days are summarized in Table II. A preliminary F test with the least significant difference, *FLSD*, as recommended by Carmer and Swanson (1971), was used to detect differences between means. The means for the pressures were significantly different ($p < 0.05$) but the variation for a specific pressure from day to day was not significant. The coefficients of variability (C.V.) ranged from 1.3 to 5.6%. We concluded that this degree of variability in emulsion preparation and evaluation was acceptable and that differences in stability could be attributed definitely to the variable being studied.

Effect of Rapid Cooling after Homogenization. Two series of emulsions containing 9% NFMS in the aqueous phase were prepared at 60 °C using homogenization pressures from 500 to 2500 psi. Stability indices of the control series were determined as described above. The second series of samples was held in 12-mL centrifuge tubes for 45–60 min at 3 °C in a water bath, and then centrifuged at 3000 rpm for the determination of stability indices.

The results in Table III show that cooling immediately after homogenization improved emulsion stability sig-

Table VI. Analysis of Variance F Values[a]

Emulsion series	1, 6, 11% NFMS	1% sodium caseinate			9% NFMS		
		RG Lecithin	Emplex	Tween 20	RG Lecithin	Emplex	Tween 20
Due to regression							
Linear X_1	0.1	87.9***	16.0*	96.5***	8.1*	45.5**	14.4*
Linear X_2	1.9	0.5	1.0	15.0*	4.1	9.9*	0.4
Linear X_3	89.1***	20.0**	3.3	0.0	0.2	0.1	3.8
Quadratic X_1^2	0.5	2.4	2.0	1.3	0.1	0.5	0.4
Quadratic X_2^2	0.4	0.9	0.1	0.0	1.6	0.4	1.2
Quadratic X_3^2	0.0	0.3	0.2	0.6	4.8*	1.2	2.0
Interaction X_1X_2	7.8*	1.2	0.5	11.0*	0.3	5.2*	2.8
Interaction X_1X_3	2.2	6.2*	5.6*	31.5**	6.3*	0.6	2.7
Interaction X_2X_3	2.6	1.7	0.9	21.6*	0.0	0.0	0.0
Deviation from regression	69.8	8.5	39.0	9.4	72.1	19.0	41.7
Coeff of determination R^2	0.95	0.96	0.86	0.97	0.84	0.93	0.85

[a] Significant F values for $p < 0.1$, 4.1 = *; $p < 0.01$, 16.3 = **; $p < 0.001$, 47.2 = ***.

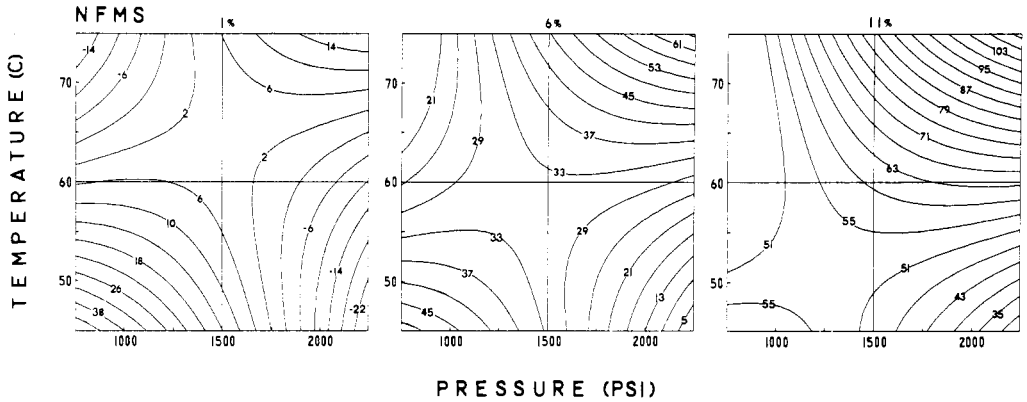

Figure 1. Response surfaces for stability of emulsions as a function of homogenization pressure and temperature. Plots left to right are for emulsions containing 25% milk fat and 1, 6, or 11% NFMS, respectively. The higher the contour number (stability index), the greater is the physical stability of the emulsions.

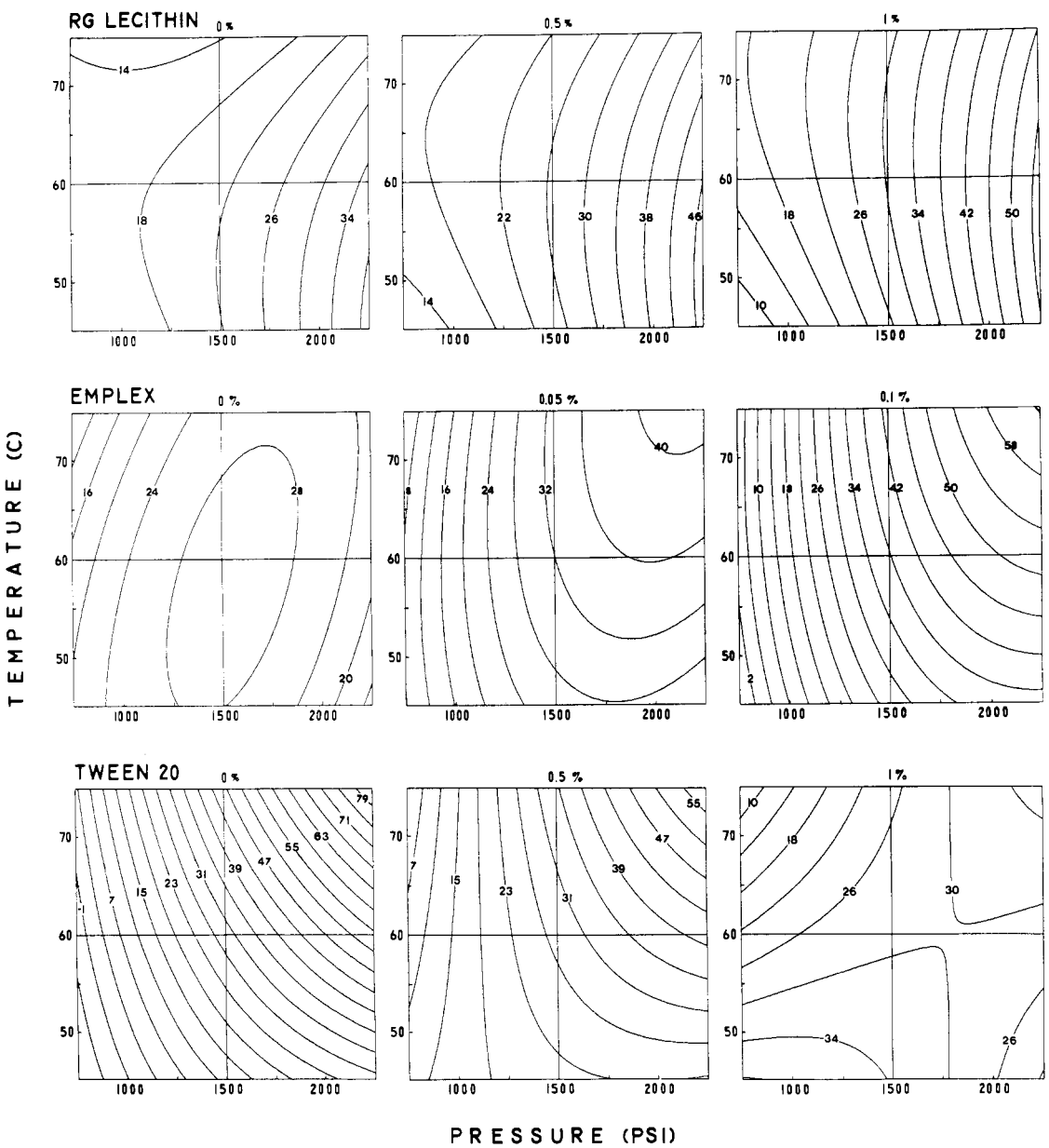

Figure 2. Response surfaces for stability index of emulsions containing 1% sodium caseinate and 25% milk fat plus RG Lecithin (0, 0.5, 1%), Emplex (0, 0.05, 0.1%), or Tween 20 (0, 0.5, 1%).

nificantly but was not as important as the effect of increased homogenization pressure. Smith and Dairiki (1975b) found previously that differences in the melting points of fats in model milk fat emulsions were less important in determining emulsion stability than the amount and kind of emulsifier employed.

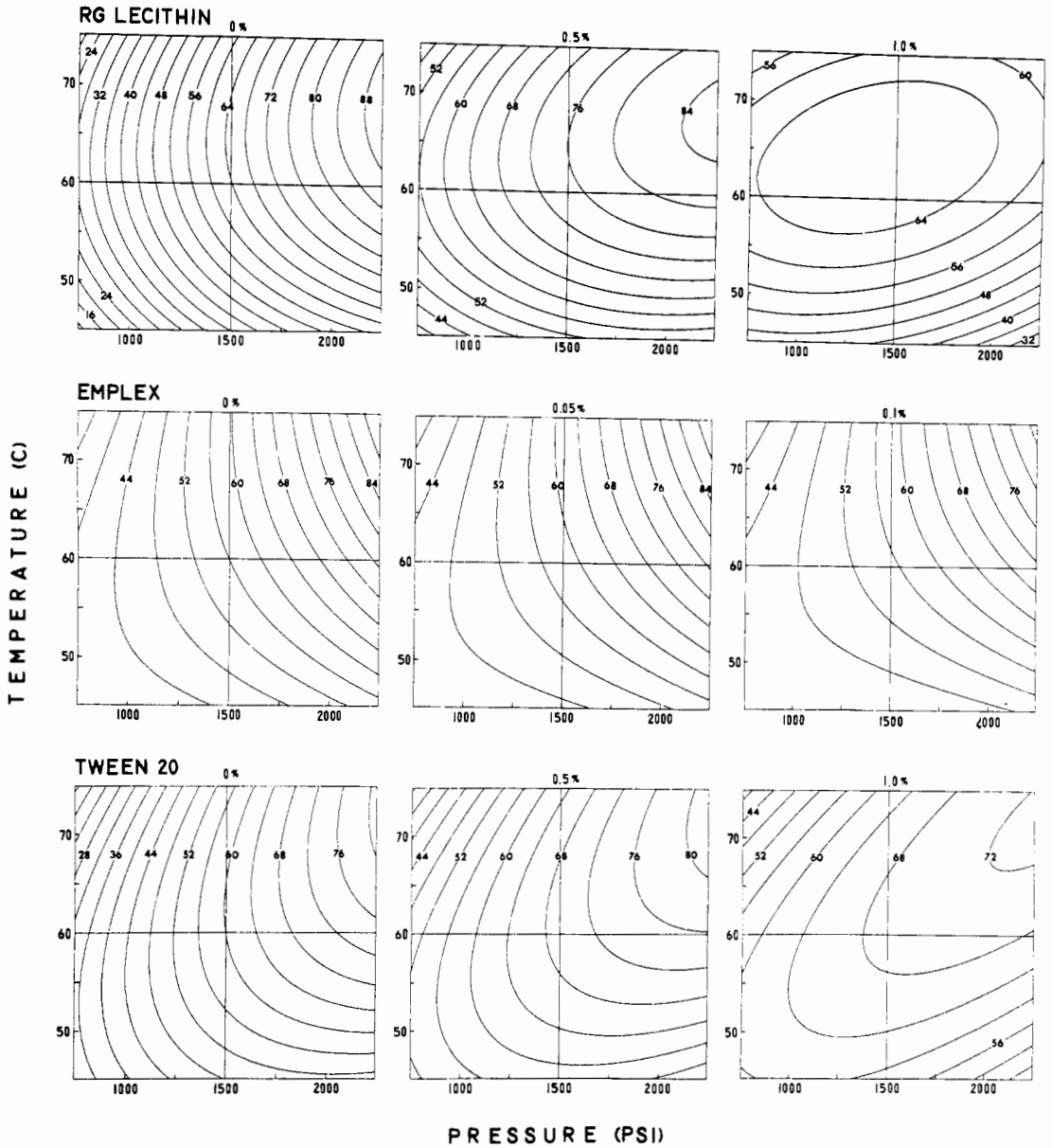

Figure 3. Response surfaces for stability index of emulsions containing 9% NFMS and 25% milk fat plus RG Lecithin (0, 0.5, 1%), Emplex (0, 0.05, 0.1%), or Tween 20 (0, 0.5, 1%).

Effect of Homogenization Temperature and Pressure on Stability of Model Emulsions. Three series of emulsions with different aqueous phases were studied using RSM. The first contained NFMS at 1, 6, and 11% levels but no added emulsifier (Table IV). The second and third series contained 1% sodium caseinate and 9% NFMS, respectively, with varying levels of emulsifier in the fat.

The experimental approach can be illustrated by considering the results for a representative experiment with emulsions containing NFMS (1, 6, and 11%). Treatment combinations and the response (stability index) are shown in Table IV. The multiple regression equation obtained from the stability index data is:

$$Y = 32.39 + 0.93X_1 + 4.12X_2 + 27.88X_3 - 2.83X_1^2 + 2.73X_2^2 - 0.54X_3^2 + 11.63X_1X_2 + 6.13X_1X_3 + 6.74X_2X_3$$

where Y = estimated stability index, and the values of the independent variables are coded (−1, 0, +1). The equation summarizes the experimental stability index results and provides the best-fitting response surface. It may be used to estimate the stability indices for variable levels not actually tested in the experiment, providing they are in or near the ranges actually used.

Analysis of variance for the experiment is given in Table V. The linear mean squares are measures of the variation accounted for by the linear component, and only the NFMS variable (X_3) is significant. The quadratic mean squares are the variation accounted for by the squared terms in the equation, and these are not significant. The interaction mean squares are the variation accounted for by interaction between variables, and only the interaction of pressure and temperature (X_1X_2) is significant. The deviation from regression mean square is a measure of the success of the polynomial equation to describe adequately the experimental results. The equation accounts for 95% of the variation in stability index, since the coefficient of determination (R^2) equals 0.95.

The response surface plots for the NFMS regression equation are shown in Figure 1. Each plot represents the response (stability index) obtained as a function of homogenization pressure (X_1) and temperature (X_2) at a

specified level of NFMS (X_3). All terms in the preceding equation were used whether significant or not, as recommended by Hader et al. (1957). In each of the three plots, there are many combinations that would fit on the same response curve. Comparison of the three plots shows that increased NFMS improved emulsion stability. At each NFMS level, there was interaction between homogenization pressure and temperature to give a minimum–maximum or saddle type model. For example, with the 11% NFMS plot, as the temperature is increased from 60 to 70 °C, the pressure giving a stability index of 63 decreases from 1800 to 1250 psi. Levels of each of the three variables can be selected from the plots to give a desired emulsion stability.

The data for the other experiments in the three series of emulsions with different aqueous phases were analyzed as above. F values from analyses of variance are summarized in Table VI, and the RSM plots for the 1% sodium caseinate and 9% NFMS series are given in Figures 2 and 3, respectively. A variety of interrelationships between variables are evident from the different forms of the curves (rising or falling ridges, bull's-eyes, saddles).

Sodium caseinate is included in many formulated foods. The level (1%) used in this research is typical of commercial simulated milk and milk products. In Figure 2, the plots for RG Lecithin and Emplex show that stability was improved by increasing the emulsifier levels. With the Emplex emulsions there was greater interaction between temperature and pressure than with the RG Lecithin series. Comparison of the three plots for Tween 20 shows that although higher temperatures and pressures increase stability, the addition of 0.5 to 1.0% emulsifier reduces stability. This suggests that Tween 20 would not be suitable for formulated food emulsions containing the levels of sodium caseinate and milk fat employed in the present study.

The aqueous phase of the third emulsion series contained 9% NFMS. This level was chosen because it corresponds to the amount of NFMS in normal bovine milk. The plots for RG Lecithin and Tween 20 in Figure 3 show that a given stability index could be obtained at lower temperatures or pressures when 0.5–1.0% of either emulsifier was added. The Emplex plot also showed nonlinear relationships between temperature and pressure, but the amount of emulsifier added had no appreciable effect. In both Figures 2 and 3 the three 0 level graphs on the left are not identical as might be expected at first glance, because each is derived from a different regression equation which best fits that particular set of data.

In summary, RSM provides the investigator with a more efficient procedure to study the numerous factors affecting specific properties of food emulsions such as physical stability, flavor stability, viscosity, texture, etc. Results reported here are limited to only one response (stability index) as affected by emulsion composition and two processing variables. Each graph provides information on relationships between variables in terms of the response so that a desired or optimum stability can be selected on the basis of cost, convenience, market requirements, etc. With the increasing availability of computers, RSM provides an important tool in biological research and food product development where several variables may interact upon each other and upon one or more responses.

ACKNOWLEDGMENT

The authors thank Central Soya, Foremost Food Company, Patco Products Company, and Western Dairy Products for gifts of materials used in this investigation.

APPENDIX: Computer Plotting Using Response Surface Methodology and UCDRSM Program

Michael B. Carter, Lloyd M. Smith, and William A. Williams

Response surface methodology (RSM) produces regression equations involving a response variable and two or more independent variables. Response surfaces generated from the equations are easy to examine and interpret when in the form of contour plots of the response variable vs. the independent variables taken two at a time. Computer plotting equipment makes possible the production of such graphs inexpensively and of publishable quality.

We have devised a three-step procedure that expedites the contour plot production: (1) obtain response surface equation; (2) develop plot on scope-type (CRT) computer terminal; (3) transfer plot to a hard copy computer plotting unit.

The UCDRSM program that we developed uses a two or three independent variable, response surface equation which includes linear, quadratic, and cross product terms. The best fitting equation may be obtained from a multiple regression algorithm available as standard software at any computer center (e.g., BMD03R, MINITAB, or SPSS-REGRESSION programs).

A contour plot of the equation is produced by UCDRSM on a scope-type terminal with line graph capability such as a Tektronix 4010. The rapid plotting capability of a scope terminal allows one to make several trial plots with different intervals and numbers of contours to attain good surface definition. Contour intervals can be uniformly or variably spaced, axes can be interchanged, graph boundaries can be changed, and different values of a third independent variable may be introduced at will on the four-compartment, square grid base. The nine intersection values of the response variable allow easy choice and identification of contours.

After a desired plot is obtained, the computer is directed to transfer the plot to a plotting unit such as a Calcomp plotter to produce a hard copy graph, usually of a quality suitable for publishing. The resulting graphs have a much higher contour resolution than line printer plots.

Two other modes of obtaining plots are available, teletype and batch. The teletype version uses any type of remote terminal for input (free format) and requires a plotter and a printer for output. Hard copy graphs are generated automatically and ancillary information is produced on the line printer. Batch input is done by free format information on cards or card-image records. Output is the same as for the teletype version.

The UCDRSM program is written in Fortran. [A program card deck (EBCDIC) and a user's manual are available from: Professor Lloyd M. Smith, Department of Food Science and Technology, University of California, Davis, Calif. 95616.]

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Received for review August 25, 1976. Accepted December 21, 1976. This work was presented as Paper No. 8 at the 30th Northwest Regional Meeting of the American Chemical Society, Honolulu, Hawaii, June 12–13, 1975. The authors thank the Dairy Council of California for financial assistance.

Protein Nutritional Quality of Florunner Peanut Meal as Measured by Rat Bioassay

Josephine Miller* and C. T. Young

Maximum growth rate of weanling rats was obtained with diets containing peanut meal as the sole source of dietary protein. The meal was made from blanched peanuts of the Florunner variety by cold expression and solvent extraction of the oil. All post-harvest treatment and handling conditions were selected to minimize changes in protein quality. Growth of rats fed 16.7 and 20% peanut protein was essentially equivalent to that of animals fed 12 to 24% casein protein. With 13.3% peanut protein in the diet, methionine, lysine, and threonine were equally limiting in the peanut meal as measured by rat growth and protein efficiency ratio (PER) of amino acid supplemented diets.

Peanuts are grown as an oilseed crop in many developing countries of the world. The defatted residue, or peanut cake, is an abundant and inexpensive source of protein readily available in some areas where protein deficiency is widespread. Unsanitary practices in many of the oil mills currently preclude use of the peanut cake for human or animal consumption, but such practices could be altered. Most of the peanuts produced in the United States are marketed for food uses as full-fat products. Some nuts are crushed for oil, however, and this might become a more significant outlet for the crop if production regulations were altered. Thus, an economical utilization of the residue remaining after oil extraction from peanuts might be beneficial both financially and nutritionally.

The protein of peanut meal is considered to be of low quality because several of the essential amino acids are present in low proportions compared with total protein. Estimates of the biological value of peanut protein relative to that of reference proteins usually fall in the range of 50 to 75%, whether evaluated by the slope–ratio technique (Hegsted et al., 1968) or by protein efficiency ratio (PER) (Neucere et al., 1972). Such tests are designed to assess the growth-promoting effects of proteins when they are present in the diet in growth-limiting concentrations. These do allow comparison of nutritional quality of one protein with that of another, but they give very little insight into the potential capacity of a protein to support an acceptable rate of growth.

The use of high dietary levels of proteins with unbalanced amino acid composition for growth of chicks, pigs, and rats was discussed by Carpenter and de Muelenaere (1965). They concluded that, under certain conditions, higher levels of poor proteins would result in nearly as good growth as could be obtained with practical diets containing good-quality proteins. However, in the studies reported, these authors used "groundnut flour (plus lysine)". They gave no details of the source or conditions of preparation of the flour nor of the lysine supplement.

Wethli et al. (1975) investigated the possibility of using groundnut meal without amino acid supplements as a source of additional protein in cereal-based diets of chicks. They reported that maximum growth rate could not be attained with groundnut meal even when very high dietary protein levels (43%) were used. Their conclusion was that the amino acids supplied by low-quality proteins were in such disproportion, compared with the animal's needs, that utilization of the first limiting amino acid(s) is impaired. Amino acid and aflatoxin content of the groundnut meal was reported.

Lysine and methionine (or total sulfur amino acids) are generally considered to be the most limiting amino acids in peanut protein for humans (FAO, 1965), chicks (Wethli et al., 1975), rats (Carpenter and Anantharaman, 1968), and rabbits (Spreadbury, 1974). McOsker (1962) evaluated the limiting amino acid sequence in raw and roasted peanuts using rats as the test animal and found threonine to be limiting also. In unroasted peanut paste, lysine, methionine, and threonine were equally limiting while in paste from roasted nuts the limiting sequence was lysine, threonine, and methionine. Calculations based on amino acid content of the peanut protein and rat amino acid requirements indicated that threonine should not be limiting and thus McOsker concluded that about 30% of the threonine in peanut protein is not biologically available to the rat. Young et al. (1973) determined amino acid content of 16 varieties of peanuts. By comparing their data with FAO (1965) recommended levels they concluded that, in addition to the three amino acids already mentioned, isoleucine and valine might also be limiting.

The source and history of peanuts and peanut meal used in estimates of biological value of peanut protein are not given in many of the studies reported (e.g., Hegsted et al., 1968; Carpenter and de Muelenaere, 1965; and Wethli et al., 1975). Such factors as variety, maturity, post-harvest drying conditions, temperatures attained during transportation and storage, and defatting processes can affect protein quality of peanut meal. In the experiments reported here post-harvest deterioration of peanut protein quality was minimized and peanuts of known variety and

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